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STUDIES ON EXPERIMENTAL STREPTOCOCCAL MASTITIS

VI. Histological Examination of the Teats of Affected Goats

By

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INTRODUCTION

The fifth paper in this series of studies on experimental streptococcal mastitis (Pattison, 1951) described the histological changes in the mammary tissue that followed the introduction of living *Str. agalactiae* strain S 13 into the teat canal of goats. This present paper completes that examination by describing the changes noted in the teats.

MATERIALS AND METHODS

The teats examined were obtained from 29 animals inoculated into one side of the udder with heat-killed or living streptococci. Details of the inocula used and of the time between inoculation and destruction are given in Table I. After fixation, teats were halved dorso-ventrally through the length of the teat canal and teat cistern. In the case of the inoculated sides, four blocks of tissue were taken from each half-teat (i.e. eight blocks from the entire teat); two blocks were taken from each half-teat of the non-inoculated sides. The blocks were taken from the same positions in all teats, and these are shown as points A, B, C and D in Fig. 1. The method of preparing sections was the same as that used for mammary tissue and described in the paper referred to above. Sections were stained by haematoxylin and eosin.

RESULTS

The Teat of the Normal Goat

The anatomical structure and histological appearance of the goat's teat have been described by Turner (1939), and examination of the teats in this series of animals confirmed his findings.

There was a wide variation in the size and shape of the teats, and no shape was more commonly encountered than any other. The dimensions of the cistern varied greatly. Two supernumerary teats were present in one animal, and in another case the teat of one side of the udder had two canals opening into two cisterns completely separated by a thick septum (Fig. 2).

The epidermis of the teat was of usual structure and similar to that of the cow (as described by Venzke, 1940): slight variations in the thickness of the layers making up the stratified epithelium were noted from animal to animal. At the external entrance to the teat canal the stratified epithelial and cornified layers were greatly thickened and continued thus through the length of the canal. At the internal end of the teat canal there was an abrupt change from

stratified epithelium to the two-cell epithelium lining the cistern (Fig. 3). Turner noted in the goat's teat, at the junction of the teat canal with the cistern, a "rosette" similar to that present in the cow; this region was characterised histologically by papillary projections (Fig. 4). In some animals the lining of the cistern was smooth and flat (Fig. 5), but in others there were numerous papillary projections (Fig. 6).

Venzke reported that the corium of the bovine teat was devoid of hair follicles, sweat glands and sebaceous glands. In the goat, by contrast, as noted by Turner, all these structures were present. Venzke also found numerous lymphocytes in the tunica propria of the cow's teat. Considerable numbers of these cells were present in a similar position in the teats of many goats.

Tubulo-alveolar glands, corresponding to the so-called "accessory glands" found in the teats of some cows (described by Venzke and others), were present in 18 of the 29 goats examined (Fig. 7). Venzke concluded that these glands secreted milk. The extent of this glandular tissue varied greatly from goat to goat; in some cases it was present even at the inner opening of the teat canal. In non-lactating goats this tissue was in an involuted state (Fig. 8), as was the mammary tissue proper, and in animals inoculated with streptococci the abnormalities of the mammary tissue proper were present also in this accessory tissue (Fig. 9). The conclusion is that this accessory tissue is true secreting mammary tissue and does not differ from the mammary tissue as a whole.

Histological Examination of Animals Inoculated with Heat-killed Streptococci

No histological abnormality was detected in the teats of four animals killed at various times after inoculation with heat-killed streptococci (see Table I).

Histological Examination of Animals Inoculated with Living Streptococci

Pattison (1951) pointed out that the degree of reaction to the inoculation of living streptococci varied from animal to animal and that the extent and intensity of the histological changes in the mammary gland showed a corresponding variation. Similar changes were found in the teats of animals inoculated with living streptococci, and in some cases only slight abnormalities were detected. Indeed, in the case of two "minimal" reactors (as defined by Pattison and Holman, 1951) killed 45 days and 54 days respectively after inoculation, no abnormality was found in the areas of teat examined, although both these animals were excreting streptococci in their milk.

In animals that developed obvious clinical mastitis after the inoculation of living streptococci (called "marked" and "intermediate" reactors by Pattison and Holman), clearly defined histo-

TABLE I

Type of inoculum.	*Time between inoculation and destruction.*	*No. of goats.*
Heat-killed streptococci	6 hours	1
,,	9 days	2
,,	45 days	1
Living streptococci	6 hours	2
,,	12 hours	1
,,	24 hours	1
,,	2 days	2
,,	3 days	1
,,	4 days	1
,,	7 days	1
,,	9 days	4
,,	13 days	2
,,	15 days	2
,,	18 days	1
,,	22 days	1
,,	27 days	1
,,	36 days	1
,,	45 days	1
,,	54 days	1
,,	63 days	1
,,	82 days	1

logical changes occurred in the teats involving mainly the lining epithelium and the tissue immediately adjacent to it. These changes were closely similar to those noted in the larger ducts of the mammary tissue proper (Pattison, 1951). No abnormality of the teat epidermis or dermis or of the sweat or sebaceous glands was found in any animal.

The earliest reaction to the inoculation of living streptococci was dilatation of lymphatic vessels and a concentration of cells, the majority neutrophils, beneath the lining epithelium of the teat and in the adjacent tissue; here and there, neutrophils passing outwards through the lining epithelium were found (Fig. 10). In some cases these changes were present six hours after inoculation.

As the reaction to the presence of streptococci progressed, macrophages and fibroblasts were added to the aggregation of cells in the subepithelial region, and the lining epithelium became hyperplastic (Figs. 11 and 12). By the third day after inoculation there was evidence of stratification of the teat-lining epithelium (Fig. 13); this stratification was usually patchy and irregular and frequently occurred in the form of small processes of epithelial cells that jutted out into the lumen of the teat (Fig. 14). In some cases stratification of the lining epithelium was apparently preceded by extensive multiplication of fibroblasts in the subepithelial region that threw the lining up into tall papillae of irregular shape. Sometimes stratification began as a patchy but rather generalised process, but in other cases it was most marked on the summits of granulation tissue projections (Fig. 15). The process continued until the greater part of the teat was lined with stratified, squamous epithelium varying in thickness from place to place (Fig. 16). Cornification was frequently found (as early as

nine days after inoculation); in some cases the cornified layer covered a wide area of epithelium, but in others it was confined to isolated projections (Fig. 17). In extreme cases the teat lining was grossly abnormal macroscopically (Fig. 18) and microscopic examination showed the presence of numerous projections of granular tissue covered by a thick layer of stratified cornified epithelium (Fig. 19).

At the height of the disease, in most cases up to about three weeks after inoculation, some degree of stratification of the epithelial lining of the teat was present in all animals. Later, when the mammary tissue proper was undergoing a process of fibrosis and involution (see Pattison, 1951), the surface layers of stratified epithelium were found being cast off (Figs. 20 and 21) until only a thin epithelial lining, usually about two cells thick, remained (Fig. 22). In the latest stages of the disease, when the udder itself was clinically atrophied and functionless, the teat lining epithelium was visually closely similar to that of the normal teat (Fig. 23). In the animal killed for examination 82 days after inoculation, small, dense collections of lymphocytes were found in the connective tissue immediately underneath the lining epithelium of the teat (Fig. 24).

SUMMARY

A histological examination was made of the teats of 29 goats inoculated into one side of the udder through the teat canal with heat-killed or living *Str. agalactiae,* and killed at times varying from six hours to 82 days after inoculation.

No abnormality was found in animals inoculated with heat-killed streptococci, nor in two animals, killed 45 and 54 days after inoculation, that gave a minimal reaction to inoculation of living streptococci.

In animals that gave an intermediate or marked reaction to the inoculation of living streptococci. there was a cellular reaction in the tissue immediately beneath the epithelium lining the teat and progressive stratification of the epithelium itself. Cornification of the lining epithelium was commonly found at the height of the disease. In the later stages of the disease the surface layers of stratified epithelium were shed until a lining epithelium about two cells thick, and similar to the normal lining epithelium, remained.

ACKNOWLEDGMENT

I am greatly indebted to Mr. F. Summerfield, Senior Technician of this laboratory, for preparing the photographs.

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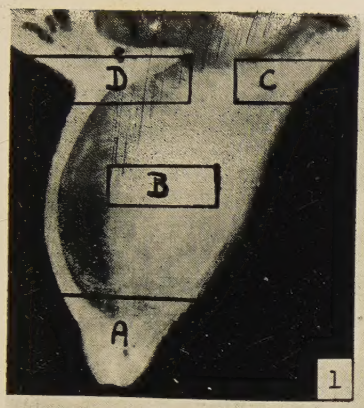
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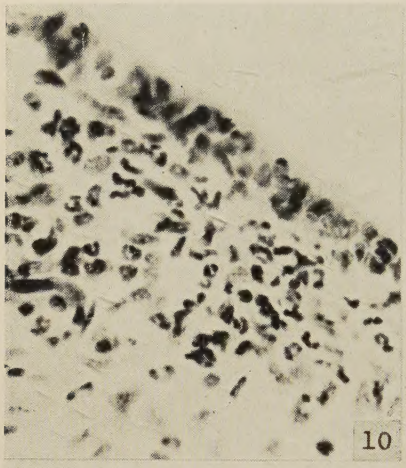
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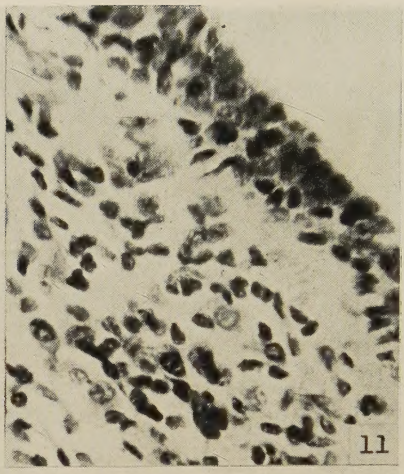
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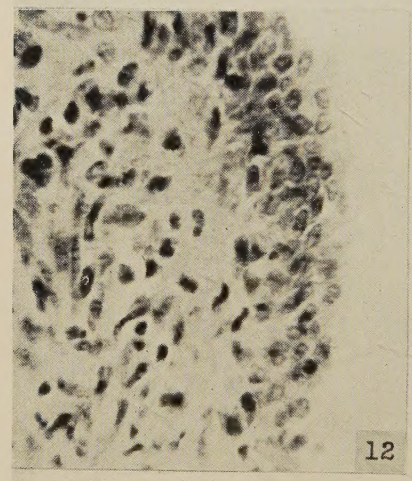
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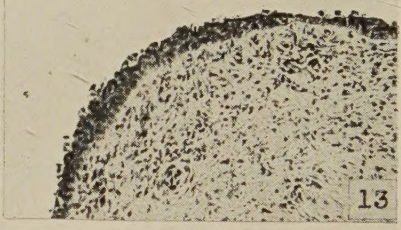
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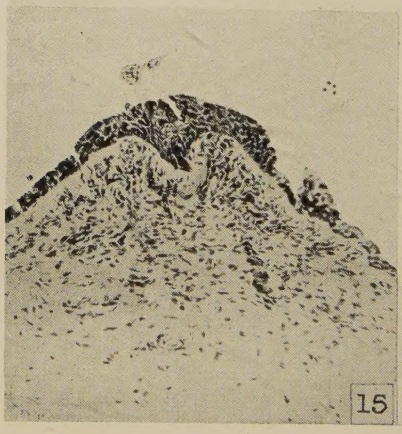
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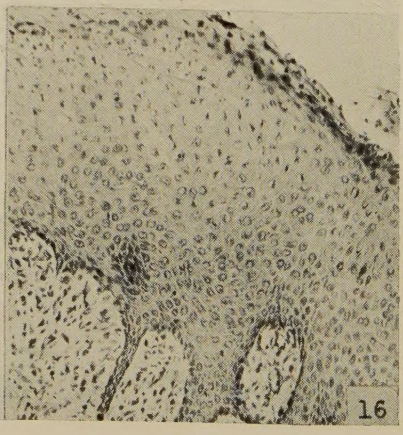
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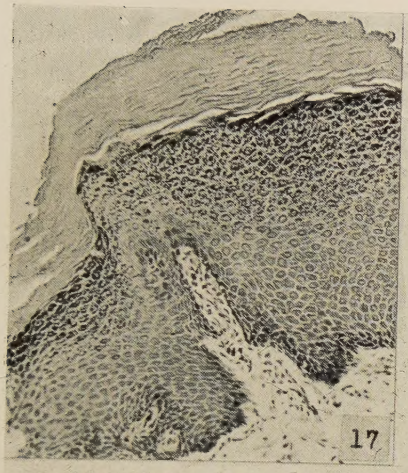
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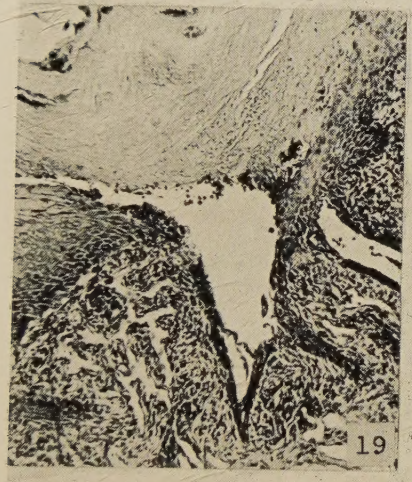
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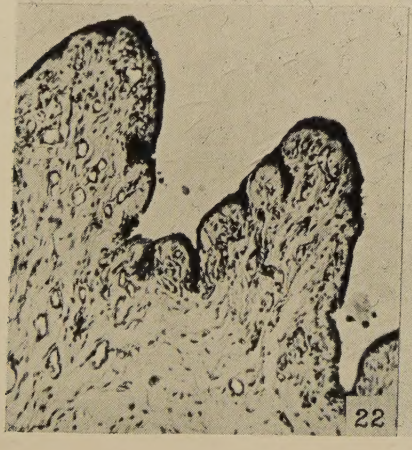
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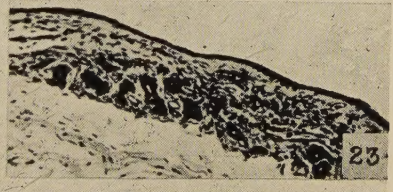
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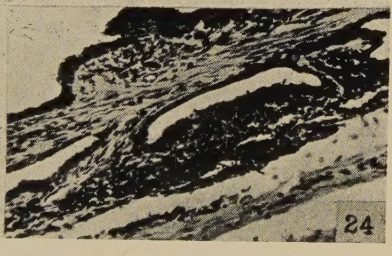
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LEGENDS FOR FIGURES.

FIGURE 1. The positions from which blocks were cut. Slightly reduced from natural size.

FIGURE 2. Teat with two canals opening into separate cisterns. Slightly reduced from natural size.

FIGURE 3. Non-inoculated animal. The abrupt transition from thick, stratified epithelium of the teat canal (top right) to the two-cell epithelium lining the cistern. H. and E. × about 70.

FIGURE 4. Non-inoculated animal. Papillary projections in the region of the teat "rosette." H. and E. × about 70.

FIGURE 5. Non-inoculated animal. Smooth, flat epithelium lining the cistern. H. and E. × about 70.

FIGURE 6. Non-inoculated animal. Cistern epithelium thrown up into folds. H. and E. × about 70.

FIGURE 7. Non-inoculated animal. Normal "accessory" gland tissue. H. and E. × about 70.

FIGURE 8. Non-inoculated animal; late lactation. Involuted "accessory" gland tissue. H. and E. × about 70.

FIGURE 9. "Accessory" gland tissue showing the same abnormalities as the mammary gland proper. Four days after inoculation. H. and E. × about 70.

FIGURE 10. Neutrophils gathering beneath the cistern epithelium; some of these cells are passing outwards into the teat lumen. Six hours after inoculation. H. and E. × about 400.

FIGURE 11. Macrophages gathering beneath the hyperplastic cistern epithelium. Two days after inoculation. H. and E. × about 400.

FIGURE 12. Fibroblasts gathering beneath the markedly hyperplastic cistern epithelium. Four days after inoculation. H. and E. × about 400.

FIGURE 13. Early stratification of cistern epithelium. Three days after inoculation. H. and E. × about 70.

FIGURE 14. More marked stratification of cistern epithelium; small processes of epithelial cells project into the teat lumen. Nine days after inoculation. H. and E. × about 70.

FIGURE 15. Stratification of cistern epithelium on the summit of a projection of granulation tissue. Nine days after inoculation. H. and E. × about 70.

FIGURE 16. A thick layer of stratified, squamous epithelium lining the cistern. Fifteen days after inoculation. H. and E. × about 70.

FIGURE 17. Stratified, cornified epithelium lining the cistern. Nine days after inoculation. H. and E. × about 70.

FIGURE 18. Grossly abnormal teat lining 27 days after inoculation. Slightly reduced from natural size.

FIGURE 19. The microscopic appearance of the teat shown in figure 18; irregular projections of granulation tissue covered by a thick layer of stratified, cornified epithelium (upper third of photograph). H. and E. × about 70.

FIGURE 20. Surface layers of stratified epithelium being cast off. Twenty-two days after inoculation. H. and E. × about 70.

FIGURE 21. Granulation tissue projections and stratified epithelium breaking down in a late stage of the disease. Eighty-two days after inoculation. H. and E. × about 70.

FIGURE 22. Late stage of the disease. The surface layers of stratified epithelium have been shed and the lining epithelium is now only a few cells thick. Sixty-three days after inoculation. H. and E. × about 70.

FIGURE 23. The last stage of the disease. The lining epithelium of the cistern is now about two cells thick and closely similar to normal lining epithelium. Note the involuted condition of the "accessory" glands. Sixty-three days after inoculation. H. and E. × about 70.

FIGURE 24. A dense collection of lymphocytes underneath the epithelium lining the cistern. Eighty-two days after inoculation. H. and E. × about 70.

ENZOOTIC PNEUMONIA IN CALVES

II. The Experimental Disease

By

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INTRODUCTION

Sporadic outbreaks of calf pneumonia which are apparently fairly numerous in different parts of the country, have been described in a previous article (Barr, MacMillan, Jennings and Kelly, 1951). The condition may be acute or chronic and calves up to 4 months of age are susceptible. The disease primarily affects the respiratory tract but may be accompanied or preceded by scours. Some cases show typical pneumonic symptoms whereas others have ill-defined symptoms, succumb rapidly and show only acute pulmonary congestion. The morbidity and mortality rates vary a good deal; in severe outbreaks the mortality may exceed 90 per cent. Recovered calves are generally stunted.

Outbreaks of a similar nature have been described by previous workers, some of whom have provided evidence that the condition is transmissible to experimental calves. These investigations have been concerned either with the etiological significance of certain bacteria which are present in the lungs in some outbreaks, or with the possible role of some other agent such as a filtrable virus.

There have been several investigations on the types of bacteria recovered from the lungs in calf pneumonia and many attempts have been made to set up the disease experimentally using cultures. Most workers are agreed, however, that no clear-cut evidence has been obtained which incriminates a bacterium as the primary etiological agent.

Carpenter and Gilman (1921) attempted to infect 23 animals either with fresh pneumonic material or with cultures. Administration of lung extract by intratracheal inoculation was successful in at least three cases, and at post-mortem there was evidence of congestion, atelectasis, and broncho-pneumonia. No attempt was made to secure serial transference of the infective agent. A diffuse purulent pneumonia was produced by a culture of *Past. septica* also instilled intratracheally.

Lamont and Kerr (1939) administered clarified lung suspensions to calves by the intranasal route. Lesions of pneumonia were found at post-mortem and in most cases a haemolytic organism was recovered from the lungs. The disease was transmitted by contact. Two calves were infected with a Seitz filtrate, but the disease was milder and the haemolytic organism was not recovered from the lungs. For this reason Lamont and Kerr postulated the combined action of a virus and a bacterium in natural cases, similar to the syndrome of swine influenza virus and *Haemophilus influenzae suis* described by Shope (1931).

Baker (1943) was able to set up a transmissible pneumotropic virus disease in mice starting with lung material from sick calves. This murine

virus was later passed to experimental calves and set up a febrile disease followed at a later stage by pneumonic lesions. No bacteria of significance were isolated from either the mice or the calves. The distribution of the virus in various tissues was determined by the inoculation of mice with calf material.

In this country many outbreaks of pneumonia, particularly in calves of about three months of age and upwards, are associated with the presence of *B. actinoides* in the lungs. Blakemore (1945) recovered this organism from acute cases of broncho-pneumonia in 6 to 12-month-old heifers and reproduced a simliar picture by the intranasal instillation of recently isolated cultures. A contact calf also contracted the disease but *B. actinoides* was not recovered from the lungs. Levi and Cotchin (1950) have confirmed the wide incidence of *B. actinoides* alone or in association with other organisms such as *C. pyogenes* in peracute, acute and chronic outbreaks of pneumonia in young bovines. The organism, which was recovered from 14 out of 26 lungs examined, failed to infect mice, guinea-pigs, rabbits or two young goats. One goat inoculated intratracheally with an infected lung suspension mixed with *B. actinoides* died 15 days later with typical broncho-pneumonia.

As earlier work on experimental calf pneumonia has been reviewed by Carpenter and Gilman (1921), Lamont and Kerr (1939) and others, it is not proposed to make any further references to the literature.

The material which served to initiate the experimental disease described in this paper was obtained from an outbreak of calf pneumonia in Cheshire. The nature of the outbreak has been described in a previous paper (Barr, *et al.*).

METHODS

Experimental calves. The calves were obtained from a number of farms in Lancashire and Cheshire and were not more than 10 days old when received at the laboratory. As far as is known no outbreak of calf pneumonia or calf scours had occurred recently on the farms which supplied the animals. In the early stage of the investigation they were kept in isolation for about 10 to 14 days in loose boxes in the experimental block before being used: moreover, as far as was possible, each experiment was terminated and the boxes disinfected before fresh calves were introduced. Later it was possible to isolate the calves in a completely separate unit where they were maintained by a separate staff and were not transferred to the experimental block until their period of quarantine was at an end. The calves were bucket fed on whole milk or on dried milk powder. Temperatures were taken twice daily.

Preparation of material for inoculation. Unfiltered material was usually a suspension of infective calf lung and turbinate in broth saline, centrifuged at 2,000 r.p.m. for 5 minutes to clarify the fluid. The supernatant fluid was used for injection. In some instances mesenteric and other lymphatic nodes were added to the suspension. Blood was collected into sodium citrate and used without further treatment or after removal of the plasma. In some

instances plasma or lung suspension was diluted to 1/100 in broth saline, centrifuged and filtered through a gradocol membrane of 0·67μ porosity.

Method of inoculation. The fluids used for the infection experiments were injected by various routes such as intravenous, intratracheal, intranasal and into the turbinate region. Sometimes a single route and sometimes a combination of two or more routes was used. The exact route of infection is indicated in the text. Except when intravenous injection alone was employed, the calves were narcotised by the intravenous injection of a suitable amount of chloral hydrate at a strength of 7·5 g./100 ml. normal saline. Fluids were instilled intranasally from a 20 c.c. syringe with the head elevated. The nozzle was inserted into the nostrils. The turbinates were reached by trephining a small hole in the superior maxilla and driving a wide bore needle into the turbinate region. In some cases the calves were chilled 24 hours after infection by drenching them from a hose.

Storage of material. Blood was collected in sodium citrate and was added to buffered glycerol (25 per cent. final concentration) or dextrose (2·5 per cent. final concentration) and stored in small MacCartney bottles at −20° C. Lungs, glands and turbinates were treated in a similar fashion.

RESULTS

Transmission Experiments

The total number of calves used in the experiments reported in this paper was twenty-one (Table I).

TABLE I

RESULTS OF TRANSMISSION EXPERIMENTS.

Nature of Inoculum	*Route*	*No. of Calves*	*"Positive" Cases*
Unfiltered Suspensions	Combined I/V, I/N, and I/ Turbinates.	12	11
Filtrate	Combined Routes	2	2
Unfiltered Suspensions and *C. pyogenes* ...	Combined Routes	2	2
Unfiltered Suspension	Intravenous	2	1
Filtrate	Intravenous	1	1
Unfiltered	Intranasal	1	0
	Contact	1	1

On the assumption that the disease was due to a pneumotropic virus it seemed reasonably certain that infection would be induced by intranasal instillation of lung and turbinate suspensions. On the other hand, we were struck by the frequent evidence of involvement of the turbinate bones and were also not unmindful of the successful transmission of influenza in the horse by the intravenous route. In order, therefore, to favour the chances of inducing infection, all the calves in the early part of the series and many of the later ones received material simultaneously by three routes, viz., intranasal instillation, intravenous injection and into the turbinate region. Subsequently the intranasal and intravenous routes were examined separately although the number of calves which could be allotted to these experiments was insufficient to permit more than tentative conclusions.

Combined Routes of Infection

Unfiltered material. Seven calves (G. 3055, J. 247, 249, 250, 279, 280, 283) were infected by the intravenous, intraturbinate, and the intranasal routes without chilling. They were killed either in extremis or at a stage in the disease when they might have died overnight or during a week-end. Two calves (J. 249 and J. 283) showed minimal lesions. J. 249 had a few small pneumonic areas in the apical and cardiac lobes together with an inflammation of the turbinates and enlargement of the retropharyngeal and mesenteric lymph nodes. J. 283 presented an acute turbinitis with congestion of the lungs. The lesions in G. 3055, J. 247, J. 250, J. 279, and J. 280 were more extensive, but followed the same pattern. The finding in J. 280 may be regarded as typical. This animal was inoculated on the 15th May, 1951 with a suspension of lung, turbinate, and blood from G. 3274. Five days later the temperature had risen to 104° F. and the calf was dull, refused to feed and was obviously very weak. There was no cough. It was killed at this stage and post-mortem examination revealed extensive areas of collapse in both lungs but most marked in the apical and cardiac lobes. The remaining lung tissue was appreciably congested and the bronchial lymph nodes enlarged and very moist. There was an acute turbinitis.

Three calves (J. 180, J. 174, and J. 223) were infected by the same three routes but were subsequently chilled. Two (J. 174 and J. 180) died four and six days respectively after inoculation.

In the case of J. 180 there was a rise in temperature to 104° F. twenty-four hours after chilling. The temperature dropped to normal only to rise again the following day. The calf was constipated and dull. Subsequently the temperature fluctuated between 104° F. and 105° F. Scouring developed on the fifth day, respirations were increased but there was no cough. The calf died on the sixth day. At post-mortem, the lesions were well marked and comprised an acute suppurative inflammation of the turbinate

bones, collapse and early pneumonic changes in the apical and cardiac lobes, and thrombosis of the smaller pulmonary veins. The bronchial and mesenteric lymph nodes were hyperaemic, enlarged and moist. The disease in J. 174 was very similar and the lesions almost identical.

Calf J. 219 was inoculated intravenously with a suspension of lungs and turbinates from J. 216 and was chilled the following day. At the end of the seventh day the animal was apparently normal and was therefore re-inoculated with the same suspension by all three routes. It was again chilled at the 24th hour. Five days after the second inoculation there was a well-marked reaction. The animal had a temperature of 105° F., there was an increase in respiration, scouring, and complete anorexia. The calf was killed. Post-mortem examination showed the usual changes in the nasal chambers and acute pulmonary congestion. There was an excess of pericardial fluid.

J. 216, which was inoculated by the combined routes with a filtrate of the lungs and turbinates of J. 190, failed to react after nine days. It was therefore re-inoculated in the same way on the ninth day after the first attempt with an unfiltered suspension of lungs and turbinates from J. 190. Six days after the second inoculation there was pyrexia and constipation. The calf was killed 15 days after the second inoculation and showed extensive consolidation of the left apical and cardiac lobes with a few early abscess areas.

There was one calf in this series which failed to react. Calf J. 223 had had no febrile reaction for four days after inoculation. It was re-inoculated with a similar suspension by the intravenous route only. The animal was killed eight days after the second inoculation and post-mortem examination revealed no gross lesions.

Twelve calves have been used for the attempted transmission of the disease with unfiltered material introduced by three routes. With one exception (J. 223), a febrile reaction and recognisable symptoms were induced, and at post-mortem examination lesions of pulmonary involvement were in evidence.

Filtered material. Two calves (J. 190 and J. 282) were inoculated with filtrates by all three routes. J. 190 received a filtrate of pooled lung, liver, brain and turbinates in dextrose and of blood, lungs and turbinates in glycerine from J. 180. The calf was chilled the day following inoculation. There was a well-marked febrile reaction, constipation, and anorexia. The animal was killed in extremis four days later. At post-mortem there was an acute inflammation of the turbinates and an early but extensive bronchopneumonia of the right apical and cardiac lobes. The lungs were very congested and varied in colour from a dark red to a yellowish grey. The cut bronchi exuded frothy fluid. The bronchial lymph nodes were hyperaemic.

J. 282 was inoculated with a filtrate of lungs and turbinates

from J. 279. It was not chilled. The calf showed little systemic disturbance apart from scouring which commenced six days after inoculation. The calf was killed 15 days after injection. At post-mortem the changes, which were minimal, comprised one small area of collapse in the left apical lobe and a few areas of congestion at the root of the lung.

Combined Routes of Infection with Superimposed Bacterial Infection.

Simultaneous inoculation. One calf (J.238) was inoculated with a pooled suspension of lungs and turbinates from J. 216 and J. 219 together with a culture of *C. pyogenes* recently isolated from a bovine lesion. Six days after infection the temperature rose to 103° F. and the calf began to scour. The temperature fluctuated around 103° F. and the calf did not thrive. It was killed eight days after infection and the following lesions were present. There was a slight suppurative inflammation of the turbinate and ethmoid bones. The surface of the lungs appeared normal but deep in the substance were areas of congestion and multiple small haemorrhages.

Delayed inoculation of bacterial suspensions. One calf (J. 237) was inoculated with the same pooled suspension of infective material as was used for J. 238, but did not receive *C. pyogenes* at this stage. A febrile reaction developed on the sixth day and diarrhoea was noted. Two days later the calf was given a suspension of *C. pyogenes* intravenously. This was followed by a series of small rises in temperature during the next five days accompanied by a loss of appetite. The calf was killed sixteen days after the initial infection. The lesions resembled those seen in J. 238 except for the absence of suppuration of the turbinates and the rather more advanced changes in the lungs. In this case, in addition to the haemorrhages and congestion, there were small areas of consolidation.

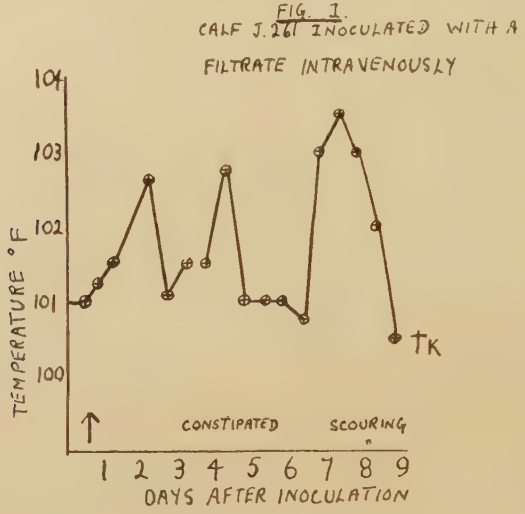
Intravenous Route

Unfiltered material. Two calves were inoculated intravenously with unfiltered material.

J. 253 was given 40 ml. of a suspension of lungs and turbinates from J. 250. The calf did not react; it was killed 16 days after the inoculation and showed no lesions at post-mortem.

G. 3274 was given a filtrate of serum from J. 261 by the intravenous route. It showed no signs of a systemic reaction during the eight days following inoculation. It was then re-injected intravenously with a pooled suspension of material from J. 253 and J. 261. Four days after the second injection there was pyrexia which subsided. On the 12th day the calf was moribund and was killed. At post-mortem examination there was an extensive area of consolidation of the right diaphragmatic lobe with slight early pleurisy. The apical and cardiac lobes showed small areas of collapse.

Filtered material. J. 261 was inoculated intravenously with 20 ml. of a filtrate prepared from the whole citrated blood of J. 253 taken during a peak febrile reaction. The calf was not chilled. The temperature curve of this case is shown in Fig. I.



The animal was dull, listless and constipated on the fifth day. Scouring commenced on the sixth day and persisted until the calf was moribund nine days after infection when it was killed. Post-mortem examination revealed fairly extensive lesions in the right apical and cardiac lobes. These lesions were mainly areas of collapse but some were pneumonic and raised. There was congestion and exudation in these areas together with compensatory emphysema at the edges. The cut bronchioles exuded frothy fluid. The bronchial lymph nodes showed little change but the mesenteric nodes were very prominent.

Intranasal Route

One calf, J. 286, was inoculated intranasally with 10 ml. of a suspension of blood, lungs and turbinates from J. 280. There was no febrile reaction at any stage, but diarrhoea commenced four days after inoculation and the calf was destroyed in a weak and emaciated state eight days after infection. The lesions comprised some congestion of the turbinates, great enlargement of the mesenteric lymph nodes and a catarrhal enteritis.

The absence of pulmonary changes may not, in this case, indicate a completely negative reaction, since an involvement of the turbinate and the mesenteric lymph nodes has been a constant feature of the pneumonic cases.

Contact experiment

A normal calf (J. 265) was placed in contact with G. 3274 five days after the latter had been infected and during its febrile stage

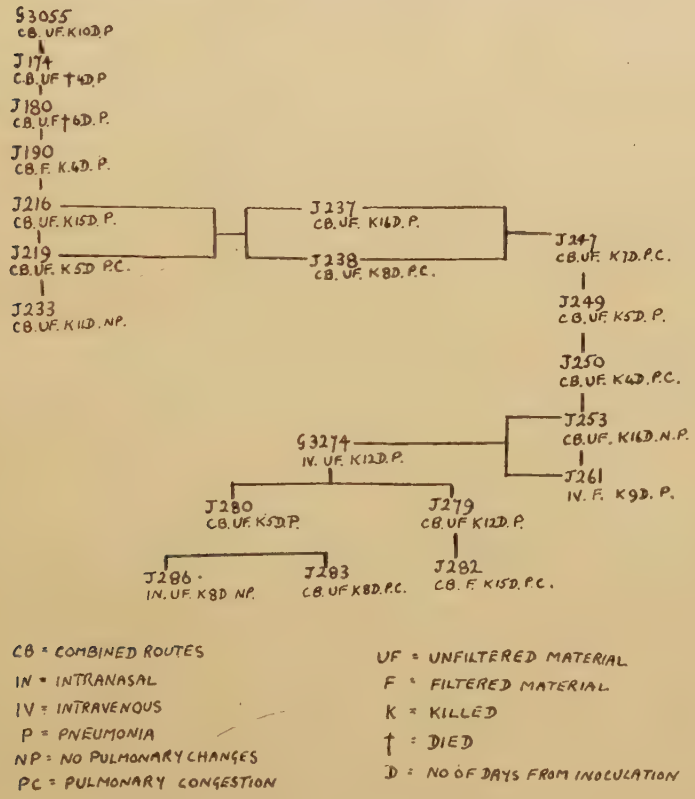
(103·6° F.). The two calves were fed from the same pail by one attendant. J. 265 had no marked temperature reactions but developed diarrhoea thirteen days after being in contact and was moribund three days later. Post-mortem examination showed a fairly extensive pneumonia spreading from the root of the lung. There was a catarrhal enteritis and the mesenteric lymph nodes were enlarged and moist.

SERIAL TRANSMISSIONS

The agent has been passed through twenty calves in series over a period of twenty months (Fig. II). On two occasions it was

FIG. II

SERIAL TRANSMISSIONS. NOV. 1949 - JUNE 1951.



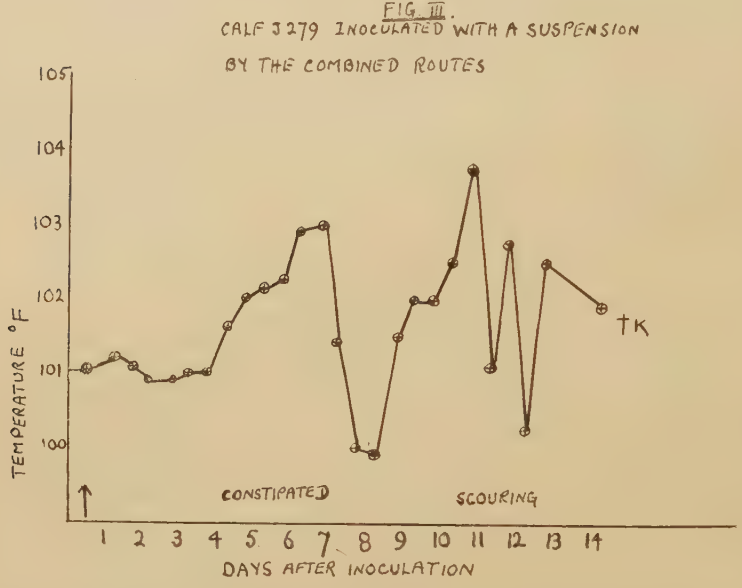
CB = COMBINED ROUTES
IN = INTRANASAL
IV = INTRAVENOUS
P = PNEUMONIA
NP = NO PULMONARY CHANGES
PC = PULMONARY CONGESTION

UF = UNFILTERED MATERIAL
F = FILTERED MATERIAL
K = KILLED
† = DIED
D = NO OF DAYS FROM INOCULATION

necessary to go back to previous passes in order to re-establish the infection. This break in the continuity of the series occurred with J. 223 and J. 261. It was not possible to determine whether this change in the pathogenicity was due to the collection of material at the wrong stage of the disease or to a reduction in virulence of the virus following storage at −20° C. We believe, however, that the first explanation is the more likely.

CLINICAL ASPECTS OF THE EXPERIMENTAL DISEASE

There were considerable variations in the severity of the clinical symptoms but in general they followed a clearly recognisable pattern. No reactions were noted until the 4th or 5th day after inoculation. Around the 5th day a febrile reaction occurred which varied in the different calves but which was never very marked. The temperature at this stage ranged usually from 103° to 104° F. The temperature of a typical case is shown in Fig. III.



There was usually some anorexia and constipation at this time. Calves which survived began to scour about the 6th to 7th day after infection and the appetite was fickle. The animals gradually lost condition and some became weak and were unable to stand. A few cases showed a very slight nasal discharge and in two calves (J. 237 and J. 261), there was acute congestion of the nasal mucosae and the conjunctivae. Coughing was not a feature of the condition. Detailed records of the pulse and respirations were not made in every case but in the more severe cases there was some respiratory embarrassment.

PATHOLOGICAL CHANGES

In a preceding section, the description of the pathological changes in the lungs has been restricted to those features which seemed necessary to complete the account of the transmission experiments. It is now proposed to give a brief survey of the findings of previous work and to compare the results with those obtained in the present investigation.

Carpenter and Gilman (1921) distinguished three main types of pneumonia in calves. They described an acute congestive form, a catarrhal pneumonia and a suppurative broncho-pneumonia. Pleuritis was often a feature of the last two types. Thorp and Hallman (1939) investigated the pathology of 40 cases of calf pneumonia and also noted three variations. The first was described as an acute exudative type of disease in which the alveoli were filled with a sero-fibrinous exudate; the second was an acute form of pneumonia which appeared to follow a chronic bronchiolitis and the third was a type in which suppuration was a constant feature. The pneumonia in these 40 cases was consistently bilateral and appeared to begin in the apical lobes, but the lesions on the left side were thought to be older than those on the right. Damage to the bronchial epithelium was a feature of the cases investigated. Lamont and Kerr (1939) noted that the pneumonia affected mainly the anterior lobes.

Sholl (1935) reported a well-marked pneumonia in calves 1 to 10 days old. There were prominent changes of congestion and cellular infiltration. The alveoli and bronchi became filled with exudate, and some parts of the lung showed dense areas of mononuclear cell infiltration.

Thorp, Shigley and Farrell (1942) examined 24 cases of pneumonia and described a typical broncho-pneumonia with marked damage to the bronchioles; some cases showed suppuration. They noted numerous giant cells and macrophages in cases which showed extensive necrosis of the mucous membranes of the bronchioles.

Langham, Thorp, Ingle and Sholl (1942) observed a bilateral broncho-pneumonia in the apical, cardiac, intermediate and anterior parts of the diaphragmatic lobes. The lesions were said to be patchy, and red and grey in colour. Collapse and emphysema were present. There was often a pleuritis. These authors considered that the disease began in the small bronchioles and adjacent alveoli. There was a cellular exudate, the bronchial epithelium was swollen and vacuolated and in places it was necrotic and desquamated. There was a peri-bronchial infiltration and an exudate which ranged from serous to croupous. Some of the vessels of the lungs showed inflammatory changes in all three layers and there was evidence of thrombosis.

Baker (1943) described a bronchitis in which the exudate was more fluid than cellular. There were vacuolated cells in the bronchi. Alveoli adjacent to the bronchi were often collapsed; their walls were thickened and some alveolar spaces contained leucocytes and red cells. In the early experimental cases well-marked lesions were not present. The changes comprised raised red patchy areas of congestion. Later cases showed consolidation of the anterior parts of the lung and a few scattered areas around the hilus. There was often slight reddening of the trachea, a few haemorrhages in the larynx, and a mucoid exudate in the trachea and bronchi, together with slight enlargement of the bronchial lymph nodes.

Gross Changes in Experimental Animals

Lungs. In the majority of cases the lesions were confined mainly to the right side and were lobular in distribution. The apical lobe appeared to be the most frequent site, followed by the cardiac lobe and, to a much lesser extent, the diaphragmatic lobe. The changes present varied from small areas of collapse and emphysema to more extensive areas of suppuration and consolida-

tion; in a few instances there was complete consolidation of the ventral areas of the apical lobe. The remaining lung tissue showed patchy congestion and in a few cases there were multiple haemorrhages. On incision mucopurulent material could, in some cases, be squeezed from the small bronchioles and in one calf there was a good deal of oedema. The larger bronchi showed a remarkable absence of change. Examination of the vascular "tree" of the lung revealed, in a number of cases, thrombosis of some of the larger veins.

The bronchial lymph node in most cases was enlarged and oedematous. The mediastinal lymph nodes showed little variation from normal except in those instances in which there was suppuration in the lungs.

Nasal chambers. In all cases the turbinates and the ethmoid bones showed acute congestion and exudation. In several cases, e.g. J. 238, small abscesses were present in the turbinate bones.

Other organs. The only constant lesion in these cases was an excess of pericardial fluid. This was a clear, slightly yellowish fluid. Some cases showed enlargement of the retropharyngeal lymph nodes. A few calves had a catarrhal enteritis and the mesenteric lymph nodes were enlarged and oedematous.

Microscopic changes

Lungs. It was possible to trace the sequence of events and it is proposed to deal with the changes in the order in which they were thought to appear.

The earliest change, detected 4 days after inoculation, was an acute congestion of the vessels and of the alveolar capillaries. At this stage there was extensive diapedesis and some haemorrhage into the alveoli, accompanied by some oedema and exudation. There was thrombosis of some of the pulmonary veins and the interlobular septa were distended with fluid which tended to infiltrate and raise the pleura. During the stage of active congestion, there was a type of collapse of some parts of the lung, which is of interest since sections failed to show any occlusion or other changes in the bronchioles which might account for this lesion. Some bronchioles did show catarrh but it was not extensive at this stage.

Following the congestion, the alveolar walls became thickened and cells appeared in the alveoli. They were scanty and were mainly mononuclears and neutrophils. The lesions appeared to be spreading by direct contact. At a later stage the cellular invasion increased in intensity, and the alveoli became filled with mononuclear cells and with exudate which was relatively free from fibrin. Areas of compensatory emphysema appeared around the affected tissue.

In the more advanced type of case with suppuration, the picture became the familiar one of a suppurative broncho-pneumonia. The small bronchi were filled with a cellular exudate,

the mucous membrane was damaged and in some areas had undergone necrosis. The areas of abscess formation showed central necrosis and extensive neutrophilic invasion. Early pleurisy was observed in one of these suppurative cases.

Turbinates. Despite the marked congestion noted on gross examination, sections of the affected bones showed surprisingly little change. In advanced cases there was very acute congestion of the vessels diapedesis of red cells and the damage to the mucous layer was quite extensive. There was a massive exudation of cells, largely neutrophilic in type, and some areas showed *frank* necrosis. Fibrin was very scanty.

BACTERIOLOGICAL FINDINGS

In many outbreaks of calf pneumonia, the lungs are relatively or entirely free from cultivatable organisms. In others, a variety of bacteria may be encountered, either in pure culture or as a mixture of two or more species. Thus streptococci, *Bact. coli, C. pyogenes,* Haemophilus and *B. actinoides* are amongst the organisms described by various authors. The inconclusive results which have been reported by a number of workers who have introduced cultures of bacteria by the intranasal, intratracheal, intravenous and intrathoracic routes throws considerable doubt on their primary etiological significance.

In the experimental calves, a bacteriological examination of the lungs was undertaken as a routine procedure. Usually fluid expressed from the pneumonic areas was seeded on to blood agar plates and often on to chocolate agar and 30 per cent. serum agar or in 30 per cent serum broth. Cultures were incubated aerobically and anaerobically. In the majority of cases, the media were sterile after 7 days' incubation. In a few instances, some scattered colonies developed but they were obviously due to extraneous bacteria and could be safely ignored.

C. pyogenes was recovered from the lungs of two experimental calves which were injected with bacteria-free suspensions. J. 216 showed small purulent foci in the left apical and cardiac lobes. An abundant pure culture of *C. pyogenes* was isolated. In J. 279 lesions of a similar nature but somewhat more extensive were noted, from which a mixture of *C. pyogenes* and *Bact. coli* was obtained. *Bact. coli* and *Staph. albus* were isolated from the lung of another calf (J. 249).

The turbinates were also relatively free from bacteria in most of the experimental animals, but in one case (J. 219) there were pockets of pus from which *Bact. coli* and proteus were isolated.

Two calves were used in an attempt to determine whether a combined infection with the virus and *C. pyogenes* or an inoculation of *C. pyogenes* at a later stage of the virus infection would produce a more severe disease. Calf J. 238 received virus and a

freshly isolated bovine strain of *C. pyogenes* by the combined routes. It reacted in a manner similar to calves injected with virus alone, and at post-mortem showed multiple areas of congestion in the depth of the lungs, but no evidence of abscess formation. *C. pyogenes* was recovered from the lungs and from the heart blood. Calf J. 237 was infected with virus and received a culture of *C. pyogenes* by the intravenous route 8 days later. At post-mortem there was no evidence of suppuration and *C. pyogenes* was recovered from the lungs only in very small numbers.

It is of interest that *B. actinoides* was not recovered from the lungs of affected calves, and in most cases *Bact. coli* was absent.

ATTEMPTED TRANSMISSION OF CALF VIRUS TO SMALL ANIMALS AND TO EGGS

In view of the establishment of a calf-pathogenic virus in mice by Baker (1943), particular attention has been paid to the possibility of adapting the strain with which we have been working to mice. Blood and suspensions of lung and turbinate have been inoculated under ether anaesthesia intranasally in mice whose lungs have been passed serially through 5 generations at intervals of 3 and 7 days respectively. The results were completely negative. Both adult and young mice (12 to 15 g.) have been used. The agent has also proved non-pathogenic for ferrets, hamsters, and guinea-pigs.

Filtrates from infective serum and from tissue suspensions have also been transferred to developing eggs by the intravenous, allantoic, amniotic, and chorioallantoic routes. With one exception the results have been indefinite or negative. In one series, filtered serum from experimental calf J. 261 following intravenous inoculation of eggs produced indefinite lesions in the chorioallantoic membrane. After further passes by combined chorioallantoic membrane and allantoic cavity inoculations, the allantoic fluid gave haemagglutination of both fowl and guinea-pig red cells to high titres. Chorioallantoic membrane, allantoic fluid and embryos from eggs in the 5th generation were inoculated into calf J. 278 by the combined routes. The animal showed no temperature reaction and the experiment was discontinued 14 days later. This calf, which was renumbered J. 280, subsequently proved susceptible to inoculation of material from G. 3274.

It was subsequently found, however, that the infective allantoic fluid had an antigenic composition similar to that of Influenza A (PR8) and as we had been working in the laboratory with this strain, it is presumed that the virus occurred in the eggs as a "contaminant". This fact is mentioned as additional evidence to that previously reported (Andrewes, Glover, Himmelweit, and Wilson Smith, 1944) that standard strains may turn up as laboratory contaminants under conditions which are rather perplexing.

DISCUSSION

The experimental disease in calves which is described in this paper is rather variable in its behaviour. The most constant features are a febrile reaction commencing about the fifth day after infection, constipation in the early stages followed by diarrhoea which may be profuse and at post-mortem examination a well-marked reaction of the nasal mucous membrane and the turbinates together with discrete areas of pneumonia. It would appear to differ in some respects from the condition evoked by Lamont and Kerr (1939), who noted a rise in temperature in their experimental calves commencing at the twenty-fourth hour and continuing for ten days. They do not mention any cases of diarrhoea in their experimental animals, although a yellow coloured diarrhoea was sometimes observed in natural cases. On the other hand, the symptoms and post-mortem findings in our calves show a close resemblance to those described by Baker (1943), namely a rise in temperature about the fourth day and later the appearance of "scours".

In the past there has been a tendency to regard outbreaks of enzootic pneumonia and calf "scours" respectively as separate conditions. The frequent isolation of *Bact. coli* as reported by Lovell and Hughes (1935) together with certain differences in the clinical picture would seem to justify this division: nevertheless, the evidence that well-marked diarrhoea is usually an integral part of the pneumonia syndrome in calves may lead to difficulties in differential diagnosis.

Lamont and Kerr (1939), and Baker (1943) have already demonstrated that a filterable agent is capable of setting up an experimental pneumonia. In the work presented here it has been shown that bacteria-free suspensions are capable of inducing a pneumonia in calves. The agent passes through a gradocol membrane of A.P.D. 0·67μ, but no attempt has been made to assess its size. The virus which Baker studied was transmitted from a natural case of the disease to mice and after several passages in this species was capable of evoking the typical disease in calves. Baker does not state that calves were infected by the intravenous route, but he detected virus in the blood by the technique of inoculating mice. We think that the evidence presented in our paper leaves no doubt that during the febrile crisis the virus is circulating in the blood stream and that at this stage the blood is capable of setting up pneumonia when inoculated into a fresh calf.

Many of the earlier workers on calf pneumonia were concerned with the aetiological significance of certain bacteria. Lamont and Kerr (1939) postulated the combined action of a virus and a bacterium in natural cases. In our experimental calves we feel safe in assigning a minor role to the part played by bacteria. Many of the pneumonic lungs proved to be bacteriologically sterile and

even in those cases where *C. pyogenes* was deliberately introduced, the final results, so far as could be judged, were not significantly different from those induced by virus alone. *B. actinoides* has been incriminated both as a causal agent and as a possible secondary invader in calf pneumonia, but so far we have failed to isolate this organism. Attention may also be drawn to the low pathogenicity of *B. actinoides* for calves (Blakemore, 1945) and for goats (Levi and Cotchin, 1950).

We have shown that the disease may be reproduced by a combination of the intravenous, intranasal and intraturbinate routes, by the intravenous route alone and by contact. In a single calf the intranasal route was used but the results were indefinite. For this reason we are unable to assess the importance of infection by the upper respiratory tract although we are aware that Lamont and Kerr succeeded in producing pneumonia in calves by this route. It may well be that in natural outbreaks the disease is initiated in this way. It is perhaps significant that both in field cases of calf pneumonia and in the experimental disease, a turbinitis similar to that seen in ferrets infected with influenza virus A, is a constant feature. This observation is strongly in favour of the early establishment of the virus in nasal passages.

Serial passage of the virus has been accomplished through twenty calves. Nevertheless on two occasions there was the apparent extinction of the agent after five or six generations. At this stage the reactions in the calves were slight and the lesions were minimal. On returning to earlier material, however, we were able on each occasion to recover the agent. Apart from the presence of the virus in the blood, the lungs and the turbinate bones we have no information on the distribution of the infective agent in other tissues. In respect of the blood stream invasion, the disease resembles equine influenza (Dale and Dollahite, 1939).

Predisposing factors are thought to be of great importance in the natural disease and our observations on the effects of a sudden reduction in the skin temperature are of interest. Attention may be drawn to enzootic pneumonia in sheep which in some parts of the country is particularly prevalent in late spring and often follows a sudden drop in temperature (Montgomerie, Bosworth and Glover, 1938). These authors were unable to reproduce experimental pneumonia in sheep by the inoculation of freshly isolated cultures of pasteurella isolated from the lungs but set up lesions with suspensions of affected lungs. They postulated that a filtrable virus was the primary causal factor but that external environmental conditions, in particular sudden chilling, were probably responsible for initiating outbreaks. We are of the opinion that chilling is a valuable aid to the setting up of infection. Calves which were chilled were more seriously affected and showed more extensive pulmonary lesions.

The histopathology of the early stages of the disease was unlike

that noted by earlier workers. Langham *et al.* (1942) and Thorp *et al.* (1942) described the condition as a typical bronchopneumonia which commenced as a bronchiolitis. In the present series of cases, however, the bronchioles were usually unaffected at least in the early stages. The initial reaction comprised congestion, oedema and collapse. This reaction was of special interest since it showed some resemblance to a form of foetal pneumonia described by Sholl (1935) which occurred in the first ten days of life and which was prenatal in origin. The histological changes were not quite the same, however, since Sholl described a dense cellular infiltration whereas in the experimental calves, cells were sparse in the initial stages. This difference seems to indicate that the collapse is not attributable to a relic of a prenatal pneumonia. In the absence of demonstrable occlusion of the bronchioles we can offer no satisfactory explanation of the collapse. It is also of interest that Baker (1943) stated that a peribronchial reaction was absent and that a very fluid exudate, areas of congestion and patchy areas of collapse were a feature of his experimental calf disease. Similar changes were noted in the present series. In addition, our pathological findings are, generally speaking, in close agreement with those of Carpenter and Gilman (1921), especially in regard to the type of change they classified as "acute congestive pneumonia".

SUMMARY

A transmissible disease of calves which produces an inflammation of the turbinates and a low grade pneumonia has been set up by the inoculation of unfiltered and filtered material. The disease is characterised by an early febrile reaction followed by diarrhoea and pneumonia. It is exacerbated by chilling the animals. The virus is present in the blood during the fever and when inoculated intravenously induces a pneumonia. Attempts to establish the agent in small animals and in the developing egg have so far been unsuccessful.

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THE TOXICITY OF RED SQUILL RATICIDE TO DOMESTICATED ANIMALS.

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INTRODUCTION

In recent years, attempts to combat rat infestations on farms and elsewhere have been intensified, and one undesirable result of this has been a great increase in the number of reports of deaths in domesticated animals due to ingestion of baits containing rat poisons. In particular, this laboratory has been requested repeatedly to investigate claims involving the deaths of farm animals.

Whilst it is possible to establish the presence in the alimentary canal of some common poisons, no reliable chemical tests exist for determining the presence of Red Squill in small quantities. This is of particular importance since red squill products are widely accepted as being non-poisonous to animals other than rodents; consequently they form the agents of choice when rat-elimination is to be practised around animal buildings (Danzel, 1936). The present investigation was conducted to see whether accidental poisoning with Red Squill could occur in domesticated animals, and if so whether any characteristic symptoms or post-mortem lesions were produced.

Of the many efficient rat poisons available in Great Britain, Red Squill is claimed to be safe for use on premises occupied by domesticated animals. This reputation is based on the following points: —(1) It is extremely unpalatable to domesticated animals. (2) When eaten it is vomited sufficiently quickly and efficiently to prevent absorption of a fatal dose of the toxic agent. (This applies only to those animals capable of emesis. The rat is said to be incapable of vomiting). (3) It is virtually non-lethal to some species even when eaten and retained. (4) Most domesticated animals, owing to their size, would not be able to find enough bait to provide them with a toxic dose.

There are, however, some disadvantages in using Red Squill. It is more expensive than many raticides, it must be imported and it is somewhat irritant to handle. Although hungry rats will eat the baits, survivors are very prejudiced against taking them a second time. This prejudice, which is also produced with other rodenticides, lasts at least one year (Freeman, 1951). However, female rats are at least twice as sensitive to squill as male rats (i.e. they are killed by a smaller quantity of bait), and this is an important advantage with regard to curtailing breeding within a colony (Winton, 1927a; Crabtree, Ward and Welch, 1939).

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To ensure ready acceptance of strange food or distasteful baits the premises are pre-baited for two or three days with inert base only. At the same time the buildings are cleared of any food material accessible to rats. Baits are deposited in and around farm buildings, along rat runways and at spots which the officer concerned recognises as being frequented by rats. Care is taken to avoid positions which allow domesticated animals to obtain the baits, e.g. by hiding behind tiles or bricks, and baits are not laid in positions from which they may be dislodged into animal foodstuffs. The number of baits laid varies tremendously: on occasion up to 50 or 100 baits in one locality may be needed. All baits not taken by the rats are collected by the officer who laid them after a given interval, usually 24 hours.

A comprehensive comparison of Red Squill with other raticides would be irrelevant here. For further discussion and for a detailed description of the techniques of rat baiting, the publications of Claremont (1921) and Freeman (1951) should be consulted.

The principal points investigated by the present author were the palatability and the posology of squill and the production of emesis by the drug, in so far as they reflected upon the danger to domesticated animals. Care was taken to consider these points with specific regard to the material in common use in the field, i.e. 10 per cent. squill powder in a simple base which usually consisted of a paste of bread and water, or sausage rusk and water. This is of some importance in palatability trials since previous investigators have deliberately used palatable substances as agents to mask the presence of squill. In addition, some observations of symptoms and post-mortem lesions are included in this account.

THE PHARMACOLOGICAL PROPERTIES OF RED SQUILL.

The Sea Onion (*Urginea scilla,* Steinh., or *Scilla maratima.* Linn.) exists in two recognised varieties, the Red and the White. The bulbs of both varieties contain glycosides which act upon the heart in a similar manner to those of digitalis. It is said that only red bulbs contain a potent concentration of principles which are highly lethal to rats (Winton, 1927, b; Wokes and Willimott, 1934; Freeman, 1951). F. R. Winton (1927, a) was the first to subject Red Squill raticide to scientific investigation and states that his preparations induced convulsions and paralysis leading to death in one to five days, but that there was no significant action upon the circulation or upon the gastrointestinal tract.

Separation of the raticidal glycosides from the cardiac glycosides, by chemical means, has not been achieved however, and recently Gold, Modell, Catell, Benton and Cotlove (1947) have shown that the production of convulsions in rats is a property of the whole group of digitalis glycosides. They suggest that in Red Squill the two actions differentiated by Winton are not produced by two glycosides, but by the same glycoside at different doses. In the rat, and only in the rat, the convulsant action is produced with low dosage and this is the action usually observed in the field.

Higher doses in the rat produce cardiac slowing leading to death before the nervous symptoms have had time to develop. In other species the cardiac effects are said to arise even with low doses and heart block always occurs before nervous symptoms have begun.

MATERIALS AND METHODS

There are very few reports dealing with the toxicity of red squill preparations to domesticated animals. Of these most are concerned with experiments using squill of unknown potency. Since the rodenticidal activity of different batches of squill can vary enormously it is often impossible to evaluate the data presented in the literature. Indeed many of the earlier investigators used preparations which, in the light of modern knowledge, would almost certainly have been useless as rat poisons. Such reports, if the workers were in fact using weak preparations, would tend to show that the domesticated animals tested were much more resistant than they would be if present-day squill were employed.

In this country, red squill powders are assayed for raticidal potency in the laboratory prior to field use. The powders issued usually have a potency such that about 400 mg./kg. will kill 50 per cent. of a group of male white rats (L.D. 50=400 mg./kg.).

The powder used in this investigation was described by the suppliers (Haller Laboratories) as having an L.D. 50 of 380 mg./kg. for such rats. A test on the stock rats at this Laboratory did not agree, and in view of this discrepancy a sample was submitted to Infestations Division, Ministry of Agriculture. They kindly assayed the product on their standard strain of rats and reported that the L.D. 50 was 490 mg./kg., with range 440 to 670, at $P = 0{\cdot}05$, and that the sample was suitable for field use.

A few toxicity experiments were made using an alcoholic extract of the powder. The potency of this preparation as compared with the original powder was estimated at this Laboratory. Three groups of rats were given respectively graded doses of (a) extract by mouth, or (b) squill powder by mouth, or (c) extract by parenteral injection. The results when analysed according to Finney (1947) indicate that the extract was 1·65 times as effective when injected as when given by mouth. 1 cc. of extract (orally) was found to be equivalent to 25 to 26 g. of squill powder (orally). The symptoms shown by rats receiving the extract parenterally were similar to those receiving extract by mouth and squill powder by mouth.

The experiments using domesticated animals may be conveniently considered in two sections, palatability trials and toxicity trials.

Palatability trials.

Hungry animals were offered, at weekly intervals, baits containing increasing concentrations of red squill powder until a concentration was found which produced complete refusal. This

scheme was tried on one individual animal from each of several species and when threshold concentrations for producing refusal were known, these concentrations were offered to fresh animals not previously treated with squill. Refusal would then not be subject to the criticism that the baits might have been accepted had the animal not previously experienced the unpleasant taste. Such a factor of refusal of second doses ("prejudice") is known to operate in rats. By this means it was hoped to find the lowest concentration at which the poison is unpalatable. If this proved to be very low it would greatly strengthen the supposition that the animals would refuse the drug when used at full strength in the field. All animals were unfed for 24 hours and were then offered bait at the rate of 2 per cent. of their body weight in each case. Unless otherwise stated the base consisted of bread paste (25 to 30 per cent. dry matter). Any material left uneaten at the end of 12 hours was removed and the animals returned to normal diet.

Toxicity trials.

Red squill powder was usually administered as a suspension in water. In the majority of animals a stomach tube was passed under the influence of short acting anaesthetics, and the red squill suspension was poured through the tube and washed through with water. In some ruminants and some dogs the dry powder was administered in gelatin capsules. In one animal only, the squill was given in a drench. The alcoholic extract was used in a few experiments in which it was considered necessary to study the effect of the drug without the complications of emesis and gastritis which frequently followed oral administration, especially in dogs. In these experiments the extract was given intravenously or subcutaneously, as required, injections of alcohol alone serving as controls.

Numbers of animals.

Winton (1927, a) found that in order to calculate an acceptable L.D. 50 for laboratory rats, 70 animals were necessary if they were of various breeding strains. If males of an inbred strain were used, only seven rats were necessary. With farm animals which are far more heterogenous than in-bred laboratory rats, an estimate of an L.D. 50 applicable to any species would have involved the use of impossibly large numbers particularly if results applicable to all common breeds were desired. Consequently only small numbers of animals were used. It is known that female rats are approximately twice as susceptible as male rats, to action of red squill and this difference may possibly apply to domesticated animals as well. Castrate rats, moreover, are known to respond similarly to female rats in this respect, (Crabtree et al., 1939). For these reasons female animals were used throughout these experiments with two exceptions (one pig, one cat).

In birds, no work was attempted since the ground is adequately

covered by the reports of Munch et al., (1929), Barnett, Blaxland, Leech and Spencer (1949) and Freeman (1951). The results described show that hungry birds will eat food adulterated with squill products, but that no serious effects ensue even when the sole diet for 14 days consists of food containing as much squill as field rat bait. Pigeons show no effects with 2000 mg./kg. of squill and higher doses produce emesis only.

RESULTS

Palatability trials.

The possible responses of the animals to the food offered to them were considered as falling into three classes designated in the following tables as "A," "D" and "R."

"A" denotes that the animal concerned accepted the material without hesitation.

"R" denotes that the animal refused the food material as soon as it was offered, and that none of it was eaten although it remained available during the next twelve hours.

"D" denotes that the animals turned from the food vessel showing their dislike at the first mouthful. These animals were able to overcome their dislike sufficiently to return to the food and eat a significant amount during the next twelve hours. The amounts eaten varied between 25 and 100 per cent. of the total bait offered.

Thus concentrations of squill which evoked an "R" response, are those which would probably be refused even by hungry animals. Squill concentrations which evoked a "D" response, are those which would be rejected and left uneaten by animals fed normally, but which might be eaten by hungry animals if no alternative food was available.

Pigs. Eleven growing pigs weighing between 20 and 60 kg. were used. Pig 76 was used as the "pilot" animal in that her reactions were first tested over a wide range of concentrations rising from 0·005 per cent. to 3·0 per cent. of squill. The other pigs were then used to check the reactions to selected concentrations. A procedure was adopted whereby each concentration was first tested on pigs which had not previously tasted squill. The results are shown in Table I in which the sequence of testing in each pig is shown by index numbers. Thus 0·005 per cent. of squill in bait was offered first to pig 76 and was accepted readily. Pig 117 received 0·05 per cent., on the first occasion, pig 127, 0·5 per cent. and so on.

Each pig was tested with at least two different baits and each bait was tested on at least two different pigs. It will be seen that the numbers of animals used to test each strength was as follows: at 0·005 per cent. eight pigs; at 0·05—three pigs; at 0·5—two pigs; at 1·0—six pigs; at 2·0—four pigs; at 3·0—six pigs; at 5·0—two pigs and at 10·0—four pigs.

TABLE I.

RESULTS OF PALATABILITY TRIALS WITH PIGS.

Varying quantities of squill powder offered in bread paste to fasting animals.

Identification Number	*Percentage of Squill in bait*								
	0%	0·005%	0·05%	0·5%	1%	2·0%	3%	5%	10%
71	A	A^2							R^1
72	A	A^2			D^1		R^3		R^4
73	A	A^3			D^1				R^2
74	A	A^3				R^1			R^2
75	A	A^2	D^3		D^4		R^5	R^1	
76	A	A^1	D^2	D^3	D^4	R^5	R^6	R^7	
100	A						R^1		
101	A						R^1		
102	A						R^1		
117		A^3	D^1		R^2	R^4			
127		A^3		D^1	R^4	R^2			

The responses of the pigs are indicated by letters "A", "D", and "R". "A" denotes that the animal ate the food readily; "D" denotes that the animals showed great dislike of the material but that they ate at least 25 per cent. of the total offered during 12 hours; "R" denotes that the animals completely refused to eat any of the material.

The index figures accompanying the letters are included to indicate the sequence of testing each animal, the first test being that corresponding to Figure 1, and successive tests on the same animal being indicated by 2, 3, 4, etc.

At all tests the adulterated bait was offered to one, two or three pigs, and at the same time the remaining pigs were offered unadulterated bait. In all cases the unadulterated bait was eaten readily.

From the results shown in Table I it is reasonable to conclude that, at least under conditions of sty feeding, pigs will completely refuse not only bait of the 10 per cent. strength used in the field but also baits of a strength of about 2 per cent. Further, the pigs showed a very marked dislike to baits containing between 0·05 per cent. and 1 per cent. They ate such material only when driven to do so by unusual hunger such as is unlikely to occur on farms. It is improbable that pigs will bolt adulterated food unmasticated, since many appear to detect the adulterant by smell in the first place. All the pigs in the experiment invariably dropped the first mouthful of distasteful food without swallowing.

Experiments with other species were arranged in the manner described above for pigs. In all cases animals were denied access to food for 24 hours. The results are summarised in Table II.

Goats. Unadulterated baits were accepted readily. Thirty observations employing adulterated baits were made on twelve females. Treated baits were accepted readily between 0·005 per cent. and 0·05 per cent, and were accepted with reluctance between 0·3 per cent. and 0·6 per cent. Baits containing respectively 1, 2·5, 3, 5 and 10 per cent. were completely refused.

Cows. Five cows were used. Unadulterated bread paste was

refused on all occasions but unadulterated sausage rusk paste was eaten by two cows and refused by two other cows. 1 per cent. squill in sausage rusk paste was accepted by 1 cow but 3 per cent. was refused completely. Two cows accepted 1 per cent. squill in an otherwise palatable cereal mixture.

Sheep. Forty-two observations were made on nineteen females. Unadulterated bread bait was frequently refused despite attempts to render it more palatable by drying and powdering. Bread bait containing 0·01 per cent. squill was accepted by two sheep but all other adulterated baits containing up to 1 per cent. squill were refused.

Unadulterated sausage rusk baits were eaten slowly and erratically over 12 hours. 1 per cent. squill in this bait was also eaten reluctantly but 2·5 and 3 per cent. squill were completely refused.

Cats. Sixteen observations were made on cats (seven female cats and one male cat). Unadulterated bait was accepted. Squill was detected in baits containing 0·008 per cent., 0·05 per cent. and 0·1 per cent, these baits being swallowed in small amounts with great reluctance.

At concentrations of 0·3 per cent., 0·5 per cent., 1·0 per cent. and 3·0 per cent there was complete refusal despite the addition of milk.

TABLE II.

THRESHOLDS OF PALATABILITY OF RED SQUILL POWDER.

Percentage in baits at which squill was detected by fasting animals, and percentage at which all animals showed complete refusal.

Species	*No. of Animals*	*No. of Observations*	*Disliked*	*Refused*
Pigs	11	35	0·05—0·1%	2%
Goats	12	30	0·3%—0·6%	1%
Sheep	19	42	—	2½%
Cats	8	16	0·008%—0·1%	0·3%
Dogs	3	3	—	0·3%
Rabbits	9	27	0·05%—10%	>10%
Cows	5	5	1%	3%

" Disliked " denotes that the animals ate at least 25 per cent. of the material offered but showed great reluctance. " Refused " denotes that no material whatsoever was eaten.

Dogs. Three observations were made on three females. Unadulterated bread was accepted readily but bait containing

0·33 per cent. squill was refused. In view of work previously reported (Munch, *et al.*, 1929; Barnett *et al.*, 1949) no further trials were made. These workers showed that baits containing 0·1 per cent. squill were consistently refused, although very palatable foods were incorporated.

Rabbits. Twenty-seven observations were made on nine females. Unadulterated bait was accepted on all occasions. 0·004 per cent. squill was accepted without differentiation, but at concentrations of 0·5 per cent., 0·6 per cent., 1·0 per cent., 2·2 per cent. and 3·0 per cent. some rabbits detected the adulterant and ate reluctantly. At all these concentrations, however, the majority of rabbits accepted the bait as readily as unadulterated bread. 5 per cent. and 10 per cent. baits were in some cases refused and in others eaten. On at least one occasion 10 per cent. squill was eaten as rapidly as unadulterated bait.

The rabbit was thus the only one of the species tested which accepted baits containing squill in strengths approaching those used in the field for poisoning rats.

TOXICITY TRIALS

Enteral administration

The results of the forced administration of red squill powder are shown in Table III.

Pigs. There are more claims for compensation involving pigs than other animals and as the pig has a scavenger's appetite it might be thought more likely to eat a fatal dose of poison than any other food animal. A small toxicity trial has been reported by Gwatkin and Plummer (1943) in Canada, but their observations were confined to very young pigs (body weights 18 to 30 lb.) which may be more susceptible than stores or fattening pigs.

The nine pigs used in the present experiment weighed between 65 lb. and 190 lb. each. All pigs receiving more than 200 mg./kg. by mouth died showing convulsions, except one male pig which appeared unaffected by 250 mg./kg. From these results it appears that female pigs are susceptible to doses of the order of 200 mg. per kilo or more; i.e. they are comparable to female rats. Reference has already been made to the finding that male rats are at least twice as resistant as female rats and that castrated males react like females (Crabtree *et al.*, 1939). The relative resistance of male pig 102 suggests the possibility that a similar sex difference may obtain in this species also.

Ruminants. The material was given to the ruminants in three different ways—by stomach tube, by capsule and by drench. The precise distribution of the toxic material in the various stomach compartments is problematical, but any such variation had no obvious effect upon the production of symptoms, or on the dose required. Probably the administration of capsules most closely imitates the natural ingestion of material like rat bait. After

TABLE III.

RESULTS OF FORCED ADMINISTRATION OF RED SQUILL POWDER.

Identity	*Sex*	*Dose of Squill Powder*	*Route*	*Anaesthetic*	*Result*
PIGS					
76	C	300 mg./kg.	Stomach tube	Pentothal i.v.	Died (6 days)
71	C	300 mg./kg.	,, ,,	,, ,,	Died (< 24 hours)
102	M	250 mg./kg.	,, ,,	,, ,,	Survived. No symptoms)
73	C	205 mg./kg.	,, ,,	,, ,,	Died (2 days)
101	F	215 mg./kg.	,, ,,	,, ,,	Killed in extremis (2½ days)
72	F	180 mg./kg.	,, ,,	,, ,,	Survived. No symptoms
75	C	150 mg./kg.	,, ,,	,, ,,	,, ,,
75	C	200 mg./kg.*	,, ,,	,, ,,	Died (6 hours)
102	M	175 mg./kg.*	,, ,,	,, ,,	Recovering. Killed after 2½ days for post-mortem.
CATS					
1	F	625 mg./kg.	,, ,,	Nembutal i.v.	Died (12 hours)
2	F	100 mg./kg.	,, ,,	Ether	Died (24 hours)
3	M	250 mg./kg.	,, ,,	,,	Died (24 hours)
4	F	200 mg./kg.	,, ,,	,,	Died (30 hours)
5	F	100 mg./kg.	,, ,,	,,	Survived (affected)
DOGS					
1	F	290 mg./kg.	Capsule	—	Survived (affected)
2	F	145 mg./kg.	,,	—	Survived (affected)
3	F	270 mg./kg.	Stomach tube	Pentothal	Survived (affected)
SHEEP					
45	F	150 mg./kg.	Capsule	—	No effect
46	F	250 mg./kg.	,,	—	Died (48 hours)
45	F	250 mg./kg.*	Stomach tube	Pentothal	Survived (affected)
120	C.	300 mg./kg.	Capsule	—	Survived (affected)
GOATS					
95	F	175 mg./kg.	Stomach tube	Pentothal	No effect
96	F	250 mg./kg.	,, ,,	,,	No effect
95	F	250 mg./kg.*	Capsule	—	Survived (affected)
96	F	350 mg./kg.*	,,	—	Survived (affected)
95	F	550 mg./kg.*	,,	—	Died (1 week)
96	F	500 mg./kg.*	Drenched	—	Survived (affected)
BOVINES					
25	Cow	50 mg./kg.	Capsule	—	No effect
25		100 mg./kg.*	,,	—	No effect
25		200 mg./kg.*	,,	—	Survived (affected)
25		300 mg./kg.*	,,	—	Died (5 days). No convulsions
1	Calf F.	100 mg./kg.	,,	—	Died (48 hours)
2	Calf F.	100 mg./kg.	,,	—	Died (24 hours)
RABBITS					
1	F	400 mg./kg.	Stomach tube	—	Survived
2	F	200 mg./kg.	,, ,,	—	Survived
3	F	300 mg./kg.	,, ,,	—	Died (12 hours)
4	F	400 mg./kg.	,, ,,	—	Survived
5	F	500 mg./kg.	,, ,,	—	Survived (affected)
6	F	100 mg./kg.	,, ,,	—	Survived
7	F	400 mg./kg.	,, ,,	—	Died (2 hours)
8	F	100 mg./kg.	,, ,,	—	Survived
9	F	200 mg./kg.	,, ,,	—	Survived
10	F	400 mg./kg.	,, ,,	—	Survived

F—Female. M—Male. C—Castrate.

*—Repeated dose of survivor after interval of one week or more.

administration by stomach tube and by capsule the presence of red squill could be detected in the rumen at post-mortem examination.

If the ruminant is no more susceptible than the rat, in proportion to its body weight, then safety seems assured on grounds of unpalatability and the large dose of squill required per animal. Cornevin (1887), however, reported that young ruminants were affected by eating growing bulbs. Hertwig (1901) produced symptoms and death with 30 and 60 g. of *fresh* bulbs in a cow. Assuming a body weight of 500 Kg., and correcting for the water content of the fresh bulbs, this would represent a lethal dose of the order of 24 to 30 mg. squill powder per kg. body weight. This implies either that in relation to its body weight the cow is much more susceptible than the rat, or that the fresh bulbs are very much more potent than the dried product. From the work of Algerian workers (see Danzel, 1936, 1939) the latter possibility is very unlikely. Accordingly it was thought advisable to ascertain the order of toxicity for ruminants. The results were very variable, but goats appeared to have more resistance than the other species. The calves died after relatively low doses (100 mg./kg.). The adult bovine and the sheep appeared to be affected by doses a little higher than those affecting pigs.

Carnivores. Most experiments were made using cats, but a few dogs were included. Palatability trials showed that dogs and cats are extremely sensitive to the taste of squill. It has, however, been possible to induce dogs to gulp down squill preparations in carefully prepared meat baits without taste being called into operation (Barnett *et al.*, 1949; Munch *et al.*, 1929), and it is theoretically possible for dogs to become accidentally or maliciously poisoned in spite of the protection of the palate. These previous investigators reported emesis in dogs in which squill products were swallowed or otherwise administered.

The cats appeared to be a little more susceptible than the other animals, one dying with a dose of 100 mg./kg. Moreover those which died after receiving larger doses had a shorter survival period than was noted with pigs and ruminants. The three dogs were given larger doses of squill but emesis was produced and an unknown quantity of toxic material was eliminated. Nevertheless, sufficient toxic material was retained to produce definite clinical symptoms in all three.

The implications of these results can be expressed in more practical form. In Table IV the potency of one ounce of field rat bait is considered in relation to the body weight of animals of each species examined. One ounce is chosen as the unit for comparison since it approximates to the size of the largest single baits laid by Pest officers.

From the foregoing experiments it is seen that a 15 kg. pig, a 20 kg. dog, a 12 kg. lamb and a 30 kg. calf would be susceptible to a single bait of this size.

TABLE IV.

TOXICITY OF 10% RED SQUILL BAIT

Species	*Threshold dose of squill for death*	*Threshold dose of 10% bait*	*Body weight for which 1 oz. of bait is dangerous*
Pigs ...	200 mg./kg.	2 gm./kg.	15 kg. (33 lbs.)
Dogs ...	145 mg./kg. (illness only)	1·45 gm./kg.	20 kg. (44 lbs.)
Cats ...	100 mg./kg.	1 gm./kg.	30 kg. (66 lbs.)
Sheep ...	250 mg./kg.	2·5 gm./kg.	12 kg. (26 lbs.)
Goats ...	500 mg./kg.	5 gm./kg.	6 kg. (13 lbs.)
Bovines...			
Calf ...	100 mg./kg.	1 gm./kg.	30 kg. (66 lbs.)
Adult ...	250 mg./kg.	2·5 gm./kg.	12 kg. (26 lbs.)
Rabbits...	300 mg./kg.	3 gm./kg.	10 kg. (22 lbs.)

In the fourth column are shown the body weights of animals which would be susceptible to 1 oz. of field bait.

Of the other species a rabbit of 2 kg. requires only about 6 g. of bait and a cat of 5 lb. only about 2 g. Any kid above 6 kg. would be fairly resistant.

The calculated amounts of bait, in ounces, necessary to induce symptoms or death in animals of specified weight are given in Table V.

TABLE V.

AMOUNT OF FIELD BAIT IN OUNCES NECESSARY TO POISON SPECIFIED ANIMALS

Species	*Body weight*	*Toxic dose of squill powder*	*Amount of bait*
Pigs ...	33 lb. (=15 kg.)	200 mg./kg.	1 oz. or less
Pigs ...	100 lb. (=45.5 kg.)	200 mg./kg.	3 oz.
Pigs ...	300 lb. (=135 kg.)	200 mg./kg.	9 oz.
Bovine ...	1,000 lb. (=455 kg.)	250 mg./kg.	38 ozs.
Bovine ...	100 lb. (=45·5 kg.)	100 mg./kg.	1½ ozs.
Sheep ...	100 lb. (=45·5 kg.)	250 mg./kg.	4 ozs.
Goat ...	100 lb. (=45·5 kg.)	500 mg./kg.	7½ ozs.
Dog ...	11 lb. (=5 kg.)	145 mg./kg.	¼ oz.
Dog ...	25 lb. (=11·5 kg.)	145 mg./kg.	½ oz.
Dog ...	100 lb. (=45·5 kg.)	145 mg./kg.	2 ozs.
Cat ...	4½ lb. (=2 kg.)	100 mg./kg.	1/15 oz. =30 grains
Cat ...	11 lb. (=5 kg.)	100 mg./kg.	1/6 oz. =75 „
Rabbit ...	4½ lb. (=2 kg.)	300 mg./kg.	1/5 oz. =90 „

Parenteral administration

In addition to the series of experiments in which squill was forcibly administered by an alimentary route, an attempt was made to give it parenterally to avoid the complication of emesis which occurred in carnivores after oral administration. The alcoholic extract described earlier was used for this purpose. When the potency of this preparation, in terms of the original powder, was determined in rats, the extract was then given parenterally as well as per os. No difference, associated with different routes of admin-

istration, was noted in the mode of action of the drug as far as this could be judged from symptoms and post-mortem examination. In particular the potency of the extract was similar when given orally and parenterally. (Ratio of potency, 1 : 1·65.)

However, initial experiments using cats revealed that extract given parenterally was much more potent than had been calculated using the "rat equivalents". The quantitative aspect of this experiment was therefore disregarded.

One dog, which had previously vomited after the administration of 270 mg./kg. red squill powder by stomach tube, was given extract intravenously. No effect was produced with alcohol alone, or with a dose of extract equivalent in rodenticidal activity to 16 mg. powder per Kg. Twice this dose, given intravenously on another occasion, produced emesis and profuse salivation within 90 seconds. Emesis continued violently for 15 minutes without respite and then diminished during the next hour. As long as 48 hours after injection, the ingestion of light meals elicited vomiting reflexes. It is possible that in the dog, emesis and salivation produced by squill may, in part, be of central (medullary) origin. The clinical picture of the violent emesis after injection did not suggest merely nausea due to some action on the heart or circulation.

Similar doses of extract were given to cats by various routes. Salivation and defaecation were readily produced, followed by profound depression and, in some cases, death. Emesis did not occur regularly in cats.

Intravenous injection of extract in fatal doses produced most violent muscular spasms in pigs and sheep, death occurring in a few minutes. In non-fatal doses in sheep and goats it produced only distress of very short duration with grinding of the teeth, defaecation, grunting and hyperpnoea. The action on the heart was not investigated.

It is possible that the emesis produced after the administration of squill by stomach tube may be due to chemical gastritis and, in part, to central action of absorbed glycosides. The observation that some animals died despite emesis (one cat and one pig) suggests that a considerable amount of toxic material had been absorbed before the stomach was emptied. If this view is correct it would explain why emesis cannot be relied upon to protect animals after the ingestion of squill rat bait.

GENERAL SYMPTOMS

The symptoms shown by pigs will be considered in detail. Initially the pigs became depressed, showing no interest in their environment or in food and tried to bury themselves beneath the straw. In some cases, the symptoms did not progress beyond this stage and the animals appeared normal within a further two or three days (e.g. pig 72). In most cases, within 12 to 30 hours after administration, further symptoms of the nature of inco-ordination

of locomotor reflexes developed. It first became obvious in one or both hind legs, as limb-twitching, exaggerated limb movements or simply as weakness of the limbs. Eventually these symptoms progressed until the pig could not rise to a full standing posture. If, whilst the pig was still able to rise, it was suitably prompted to move, it did so with great reluctance and showed obvious difficulty in controlling the movements of its hind legs. No signs of peripheral pain were observed. Animals in the initial stages of the nervous syndrome would show hyper-sensitivity as evidenced by excessive blink reflexes and flinching when stimulated by sound, light or movements in the immediate environment.

Recovery occurred in pigs which had reached the stages of depression and slight posterior inco-ordination, but those which progressed beyond this did not recover. In one case (pig 76) after slight symptoms of inco-ordination the animal appeared to be recovering on the third and fourth days after dosing. At the end of the fourth day the condition deteriorated, however, and the pig slowly advanced through various degrees of inco-ordination until it was prostrate two days later. It became more and more exhausted as convulsions increased in severity and it lost flesh rapidly. It was finally killed on the sixth day. Subsequently, all pigs which had reached the stage of complete inco-ordination or of convulsions, were killed immediately.

In these severe cases, as symptoms advanced the inco-ordination passed to the anterior limbs and neck. In time (usually about 24 hours after initial symptoms were noted) the pig would be unable to rise and would lie on its side often showing inco-ordinated movements of the head and limbs. In some cases, these movements were continuous and in others they occurred in violent bursts of activity, with prostration intervening between convulsions. All skeletal muscles appeared to be affected, including those of mastication and the external muscles of the eyes. Hyperpnoea was noticed in several subjects but it occurred only for short periods of about an hour or two. No significant rises of temperature occurred, and successive readings from individuals showed wide variation within a range of 101° and 104° F. No consistent changes in the heart rate were noted. Shivering was observed in many cases but was not related to temperature changes and was more probably one facet of general motor inco-ordination. Diarrhoea occurred in a single pig, which was also the only one in which emesis was known with certainty to have occurred. Emesis, although very pronounced, did not prevent the death of this animal.

Symptoms in other species were either of a convulsive nature as in the pigs, or of an intense and general depression. All affected animals developed symptoms after 6 to 16 hours, and these progressed over a varying period up to one week (goat 95). The majority of all deaths occurred between 24 and 72 hours after administration of the drug.

In ruminants in which the drug was given by stomach tube, by gelatine capsules, or as a drench, the different routes of administration may have been associated with variation in the distribution of the powder in the stomach. Nevertheless there was no obvious variation in the time of onset of the symptoms. Sheep 46 showed inco-ordination, tremors, eye oscillations, hyper-excitability and, finally, generalised extensor spasms similar to those of rats. Sheep 45 showed only mild symptoms of inco-ordination, hyper-excitability and diarrhoea. The goats, however, although showing some slight motor inco-ordination, were depressed rather than hyper-excitable, even with doses much higher than those affecting the sheep. The adult bovine and one calf were also depressed, whereas the other calf became very hyper-aesthetic and inco-ordinated, eventually passing into convulsions.

The cats showed depression to a marked extent, one appearing to pass to a profound state of collapse or shock. Only one cat showed emesis after oral administration of squill powder. Vomiting occurred in all dogs from one hour after dosing with capsules. It was accompanied by salivation and depression. The stomach was emptied by the first spasms, but nevertheless the emesis continued for five minutes and at intervals during the next six hours. All had loose faeces next day and were then depressed and shivering. Recovery was rapid and was complete within two days.

Diarrhoea after oral administration was most marked in the case of the adult bovine, but was not a cardinal symptom in any other species. There was no sustained rise or fall of temperature in any animal, although some showed remarkable swings of 3 or 4° F. over a few hours. Respiration also varied very greatly in individual animals. Short periods of rapid panting alternating with periods of quiet breathing, occurred very frequently and may have been associated with abdominal discomfort.

LESIONS AT POST-MORTEM EXAMINATION

There is very little to add to the general findings of Gwatkin and Plummer (1943) in pigs, and of Winton (1927a) in rats. The principal lesions were confined to the stomach, the intestines and the circulatory system. All parenchymatous organs examined histologically, showed, to varying degrees, early degenerations of a non-specific nature.

Pigs. Of the six pigs dying after oral dosing with red squill powder, all showed inflammation of the alimentary canal. Pig 75, which died within six hours, had only patchy hyperaemia of the stomach and intestinal walls. Pig 71, which died within 24 hours of dosing, showed developing enteritis and gastritis and one large ulcerated area in the pyloric region of the stomach wall. Pigs 73, 101, 102 and 76, which died in 2, 2½, 2½, and 7 days respectively, showed very marked congestion and ulceration of the stomach wall. The whole mucosa in each case was purple and oedematous

with areas of deep ulceration, which, in pigs 73 and 76, were fully 10 inches in diameter. In pig 101 enteritis was very slight but in 102, 73 and 76 the mucosa from the duodenum to the rectum was greatly inflamed throughout presenting a purple and oedematous appearance. The large bowel was more severely affected than the small bowel. In pig 76 the contents of the intestines were extremely haemorrhagic. In all 4 pigs surviving more than 24 hours, there was marked congestion of the mesenteric vessels, and the mesenteric lymph glands were swollen and congested. This striking prominence of the mesenteric vessels was also found in other species. In one pig sub-peritoneal haemorrhages (¼-in. in diameter) were found in large numbers on the surface of the abdominal wall. In 4 of the 6 pigs which died, the stomach contents were very watery, suggesting the secretion of large quantities of saliva or of gastric juice. Increased drinking of water did not occur. Kidney, lungs and liver in all six animals showed varying degrees of congestion, and in specimens sent for microscopical examination general degenerative changes were noted (cloudy swelling, cell fragmentation, karyorrhexis, etc.).

Ruminants. The lesions were similar to those found in pigs. The cow showed a patchy enteritis but no severe gastritis. One calf showed patchy inflammation of the small intestine, mild gastritis (abomasum), and pronounced congestion of the mesentery. The second calf could not be examined.

In the sheep and goat there was relatively little to be seen beyond a mild degree of enteritis and gastritis, inflammation of the mesenteric lymph-nodes and congestion of mesenteric veins. It appeared that although toxic material was absorbed just as efficiently in ruminants as in pigs, it did not produce in them the severe gastritis and enteritis seen in the latter species.

One sheep and one pig, given fatal doses of extract intravenously, showed an intense congestion of all abdominal organs except the spleen. This may possibly be due to a direct action of a cardiac-active principle on the circulation. In two cats given fatal doses of squill extract intravenously, there was an intense congestion of the veins leading to the heart. A similar appearance was found to a less striking degree in two cats which had died after the subcutaneous injection of extract. This is in keeping with the report made by Gold *et al.* (1947), that excessive slowing of the heart, due to the digitalis-like action of squill, occurs in the cat.

DISCUSSION

Squill must be judged in relation to the danger it presents to domesticated animals. As stated, it is thought to be safe for the following reasons: —The substance is non-toxic to poultry; it causes emesis in many animals with elimination of the toxic material; large amounts of bait must be eaten by farm animals before an adequate dose per unit body weight has been ingested; and it is unpalatable.

In these experiments red squill was shown to be toxic to pigs, sheep, goats, cats, dogs, rabbits and bovines. Emesis was observed in pigs, dogs and cats but not in ruminants or rabbits. In pigs, emesis occurred rarely but did not prevent death even with moderate dosage. In cats, it occurred in only one of five cases. On the other hand all three dogs vomited. The marked and prolonged emesis which occurred in the dog subjected to intravenous injection of extract suggested that emesis, at least in this species, is possibly of central origin and not merely a reflex from an irritated gastric mucosa. It is evident that the production of emesis cannot be relied upon to protect animals against death.

With regard to fatal dosage, the experiments give some indication of the amount of bait which a given animal must eat before suffering ill effects and possibly death. *The figures can only be regarded as a general guide.* It is worth noting that the poison appeared to have the same order of toxicity for female domestic animals as it has for female rats. The range of dosage per Kg. body weight required to produce death was much the same as that to be expected in experiments with female rats. From the results obtained it can be confidently asserted that all pigs, goats, sheep and cattle, except possibly very young animals, would not be able to find a fatal dose of poison after the laying of squill for the elimination of rats on a farm. In this connection it must be remembered that elaborate precautions are taken by Pest officers to recover baits not eaten by rats.

It is considered certain that pigs and ruminants will refuse to eat bait containing 2 per cent. or more of red squill powder. Unless very hungry they will also refuse baits containing much lower concentrations. Since the usual field bait in this country contains 10 per cent of the powder, these animals are adequately protected by the palate, for observations have shown beyond doubt that even if an animal should pick up some 10 per cent. bait, it would reject it immediately. However, these results can be applied strictly only to animals under conditions simulating those of the experiments. They would not necessarily hold good, for instance, in the case of pigs fed habitually on processed scraps (e.g. "Tottenham pudding") since such animals may have developed greater gustatory tolerance than pigs fed on meal. (Rietz and Moore, 1943; Brüning, 1937.)

Dogs and cats are at least as susceptible as other species and as they are small animals, it is possible that sufficient material could remain behind after field operations to represent for them a fatal dose. These animals are extremely sensitive to the odour and taste of red squill, however, and this alone is an adequate safeguard against the voluntary ingestion of field rat-bait.

According to Freeman (1951) cats and kittens have been observed to eat dead and dying rats on farms where squill is in use: none came to any harm. In addition, cats have also been observed

to eat the whole carcase except the stomach and duedenum of rats receiving lethal doses of red squill, thus emphasising the protection afforded by the palate. This would not of course protect dogs against malicious poisoning if sufficient care were taken to disguise the bait, for instance by secreting a fatal dose in a piece of meat which could be swallowed without mastication. Such malicious poisoning, although a remote possibility, may occur since red squill raticide is not confined to official usage, but is freely available to the public for domestic use. Early emesis in the dog may be a form of protection, but on the other hand cats have been observed to die without showing emesis.

Rabbits. These animals are not greatly protected by their palate or size. They do not vomit and as they are only slightly more resistant to squill than are rats they must be considered as unprotected against squill.

The mode of action of red squill raticide does not elicit clearly recognisable symptoms or post-mortem lesions. The great nervous stimulation seen in the rat was reflected, in these experiments, by convulsions, hyperaesthesia, or locomotor inco-ordination seen in many animals, especially the pigs. However, some animals, particularly cats, showed profound depression which progressed to coma, without any sign of nervous stimulation.

It is possible that two or more influences are at work, *viz.*, a C.N.S. stimulation and a digitalis-like action on the heart. This suggestion was first made by Gold *et al.* (1947) who declared that the typical action of red squill glycosides was like that of digitalis, but that the rat was probably a unique species in that it also showed a profound nervous response. The present experiments show quite clearly that the rat is not unique in this sense, for orally-administered red squill acted as a convulsant in pigs, goats, sheep, bovines and rabbits. Nevertheless, some evidence of circulatory disturbance, particularly congestion of the large abdominal and thoracic veins, was noted at post-mortem after oral and parenteral administration of red squill. This is in keeping with the possibility of the drug producing excessive slowing of the heart, or heart block. It is probable that in those animals which died without showing nervous excitement, death was due to interference with cardiac function.

The detailed investigation of such matters was considered as falling outside the province of the present experiments. No attempt could therefore be made to collect pertinent objective evidence.

SUMMARY

The dangers to which domesticated animals are exposed as a result of rat-elimination procedures are discussed, with special reference to Red Squill raticide.

Red squill powder is very unpalatable to pigs, sheep, goats, cats, dogs and bovines. Even at very low concentrations the poison is

detected and disliked. Baits much weaker than field bait are completely refused even when the animals are denied food for up to 36 hours.

Red squill produces toxic effects in female pigs, sheep, goats, bovines, cats, dogs and rabbits, in doses per unit body weight, of the same order as those effective in the female rat. The most resistant animals in these experiments were goats; the least resistant were cats and calves. Except for very young animals a toxic dose for any pig or ruminant would not be available after the laying of baits on a farm.

Emesis may occur in some species after ingestion of red squill but it cannot be relied upon to protect the animal.

Red squill, in addition to acting as a convulsant, may give rise to gastritis and enteritis, and may slow cardiac conduction. The principal symptoms and post-mortem lesions vary from animal to animal depending on which of these actions predominates.

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CARBOHYDRATE METABOLISM IN THE BOVINE
III. The Effect of the Administration of Glucose by Mouth to Normal Cattle of Various Ages*

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INTRODUCTION

Glucose, usually in the form of molasses, is frequently given by the mouth in the treatment or attempted prevention of bovine ketosis. The investigation reported in this paper was undertaken to obtain information regarding the possible value of this form of therapy, particular attention being given to the blood sugar level and urinary constituents following the oral administration of glucose. Observations were made on immature animals as well as adults since it was considered that the degree of development of the rumen might affect the response.

THE ORAL ADMINISTRATION OF GLUCOSE TO MATURE AND IMMATURE CATTLE

Procedure

Two $3\frac{1}{2}$-year-old cows, a Red Poll and a Shorthorn respectively, a five-month Shorthorn heifer calf and a twelve-month Guernsey heifer calf, were available. Glucose monohydrate was given by stomach tube to the adults and by mouth to the calves. The dosage ranged from 0·25 lb. per cwt, to 1 lb. per cwt., which is approximately equivalent to 2·24 g./kg. to 8·94 g./kg. Observations were based on blood samples collected after administration at 30 minute intervals for the first three hours and then every hour for the next four hours. Urine was collected when possible by perineal stimulation every 30 minutes for the first four hours and then every hour until eleven to twelve hours after dosing; a further sample was obtained after 24 hours.

The methods described in a previous paper (Holmes, 1951) were employed for urinary analysis and blood sugar was estimated by the method of Hagedorn-Jenson using Somogyi's modified protein precipitation method.

Results

The findings are summarised in Table I. In the two calves aged five and twelve months a dose of 4·47 g./kg. of glucose given by mouth resulted in a rise in blood sugar which was slightly higher and more prolonged in the younger animal, the response more closely resembling that of a single-stomached animal. (Fig. 1.)

Urine samples proved difficult to obtain from the calves. In the Shorthorn, glucose was not detected in three samples obtained

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TABLE I

SUMMARY OF THE DATA FROM THE EXPERIMENTS ON THE EFFECT OF GLUCOSE BY MOUTH.

Animal	*Age*	*Lactation (1st calf) days*	*Pregnant (2nd calf) days*	*Wt. Cwt.*	*Dose g./kg.*	*Pre-drenching Blood Sugar mg./100 ml.*	*Max. Blood Sugar Rise mg./100 ml.*	*Time of Max. Sugar Rise Hrs.*	URINE				MILK	
									Acetone	*Sugar*	pH	*S.G.*	*Yield lbs.*	*Acetone*
Shorthorn calf ...	... 21 wks.	—	—	3⅛	4·47	69	66	2·5	−ve	−ve	7·5–8·0	1·025–1·052	—	—
Guernsey calf ...	... 49 wks.	—	—	4¾	4·47	65	62	2·0	−ve	−ve	7·5–8·0	1·023–1·052	—	—
Red Poll cow ...	... 3½ yrs.	364	161	8	2·24	49	—	—	+ve	−ve	5·5–6·0	1·037–1·054	8–9	−ve
Red Poll cow ...	... 3½ yrs.	350	147	8	4·47	49	16	0·5	+ve	−ve	7·5–8·0	1·026–1·053	9½–11	−ve
Red Poll cow ...	... 3½ yrs.	357	154	8	8·94	50	111	0·5	+ve	+ve	6·0–8·0	1·014–1·038	8–11	−ve
Shorthorn cow ...	... 3½ yrs.	155	52	8¼	4·47	46	21	0·5	+ve	−ve	5·0–7·5	1·029–1·051	13–14	−ve
Shorthorn cow ...	... 3½ yrs.	162	59	8¼	8·94	50	30	0·5	+ve	−ve	5·5–7·5	1·039–1·061	11–13	−ve

0 to 3 hours after dosage but subsequent samples collected at 4, 5 and 7 hours gave a slight reduction of Fehling's solution. Quantitative estimations were not possible as only a small quantity of urine was available, but in view of the blood sugar level it seems probable that the results of the urine examinations indicated glycosuria. Urine from the Guernsey one hour after dosage was negative for glucose, but samples at 3 and 6 hours contained 1·7 g. per cent. and 1·0 g. per cent. respectively.

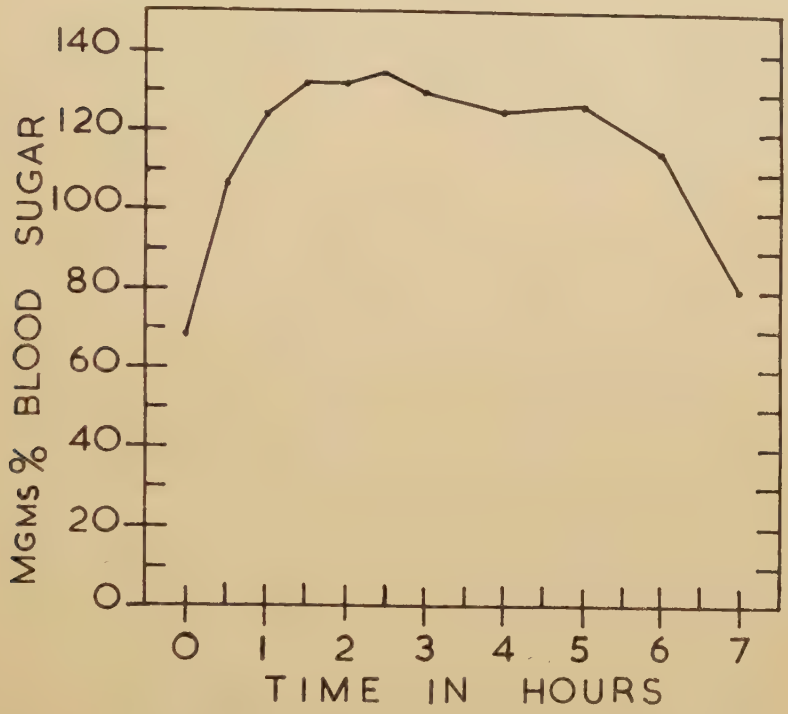

FIG. 1. Shorthorn heifer calf. Age 21 weeks. Dose: 4·47 g./kg.

In the adults, the results were different. A dose of 2·24 g./kg. given to the Red Poll did not cause a rise in blood sugar. (Fig. 2.) When double this dose was given to this animal and to the Shorthorn there was a slight rise with a return to normal within one hour. The result was somewhat different when a dose of 8·94 g./kg. was given to the same two animals. In the Red Poll there was a considerable rise in blood sugar with glycosuria, the total glucose excreted being 4 g. and it was five hours before the blood level returned to 60 mg. per cent. (Fig. 3.) In the case of the Shorthorn there was only a transitory rise and no glycosuria.

Acetonuria developed in both the cows during the course of each experiment but it was not observed in the calves.

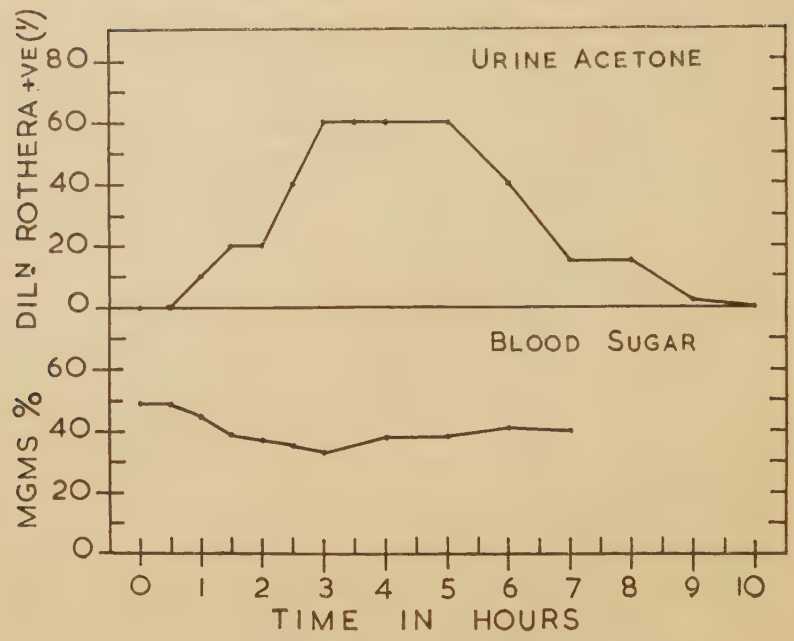

Fig. 2. Red Poll Cow. Dose : 2·24 g./kg.

THE SITE OF ACETONE FORMATION IN THE RUMINANT

It was observed that there was a time lag between the administration of glucose and the appearance of acetone in the urine. This is apparent in the results set out in Table II in which the experiments are tabulated in the order in which they were carried out. It was considered that this lag might represent the time required for the acetone to be formed from the glucose either in the rumen during fermentation of the sugar or after absorption, probably in the liver. In both animals the appearance of acetonuria was delayed by increasing the dose. It would appear that the smaller doses of glucose were rapidly dealt with in the rumen and their products absorbed. There was a delay in the appearance of acetonuria following the larger doses which may have been due to the administration of a concentration of glucose well above the optimum which caused a temporary reduction in the rate of ruminal fermentation. This argument is supported by the finding that high doses of glucose caused an increase in blood sugar which was probably due to delay in ruminal fermentation resulting in absorption of glucose as such. When a small dose (2·24 g./kg.) was given to the Red Poll not only did the blood sugar fail to rise but it became sub-normal and at the same time acetone appeared within one hour in the urine (Table II).

It has been suggested that acetone is formed in the rumen by bacterial action and in support of this suggestion Boddie (1949) found that acetone was produced when the rumen contents of

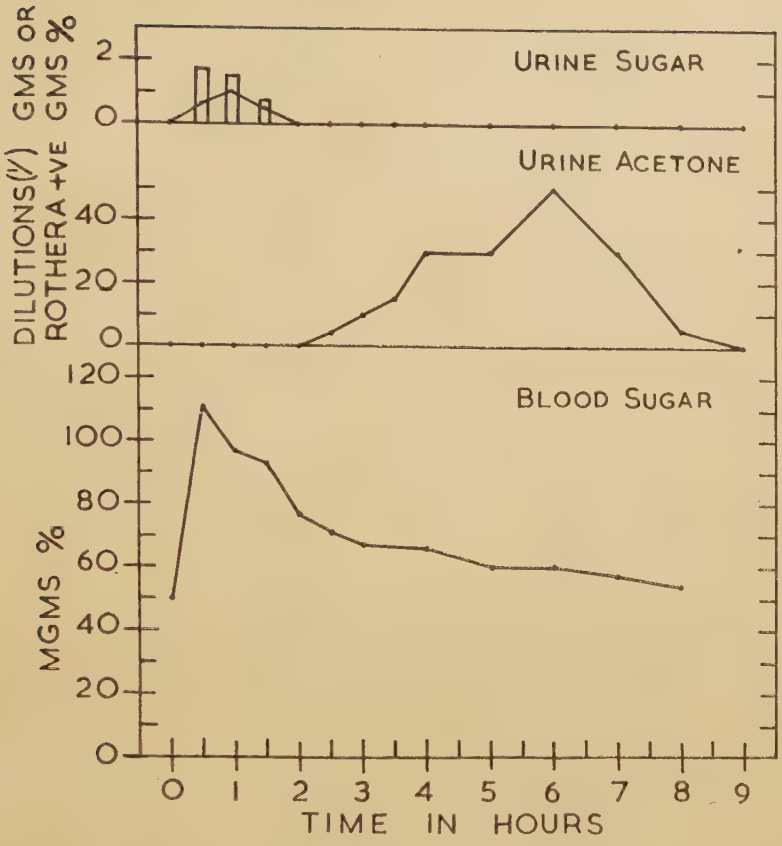

FIG 3. Red Poll Cow. Dose : 8·94 g./kg.

TABLE II

GLUCOSE BY MOUTH : URINARY ACETONE EXCRETION.

Animal	*Dose g./kg.*	*Time after dose before appearance of acetone. Hours.*	*Max. + ve diln.*	*Time of excretion of highest conc. of acetone. Hours.*	*Time of disappearance of acetone Hours.*	*Duration of acetone excretion. Hours.*
Red Poll ...	4·47	2·5	1/20	3·5	6	2·5
Red Poll ...	8·94	2·5	1/50	6	9	3
Red Poll ...	2·24	1	1/60	3	10	7
Shorthorn ...	4·47	2	1/20	4	8	4
Shorthorn ...	8·94	5	1/80	8	10	2

cows were incubated with foodstuffs such as turnips. A similar finding was reported by Duncan, Huffman and Tobbin (1939) who described the formation of acetone in-vitro by ruminal bacteria acting on cellulose. In view of this evidence it might be suggested that the results in Table II were influenced by the fact that the initial doses of glucose encouraged the growth of acetone-producing bacteria leading to greater production from the later doses. In the Red Poll cow the smallest dose was given last and resulted in the excretion of the highest concentration of acetone. On the other hand, if the liver is the organ concerned it may be that the smaller dose more nearly approached the optimum concentration of glucose that can be fermented in the rumen. A small dose might, therefore, result in an increased rate of ruminal fermentation with the absorption of larger quantities of the products of fermentation and consequently a greater production of ketones in the liver followed by their temporary excretion in the urine, when they accumulate in excess. An experiment was, therefore, devised to obtain further information as to the role of the rumen in acetone production.

Procedure

A dose of 8·94 g./kg. of glucose was given to the Shorthorn cow at the same time on three successive days. Blood and urine samples were collected at frequent intervals as indicated in Fig. 4. Rumen samples were collected each day prior to administration of glucose and at two and six hours after it had been given. A stomach tube was used for the collection of rumen samples and the ingesta was aspirated by means of a stomach pump. The material collected was usually composed of a greenish-brown fluid containing small pieces of hay. All tests were made on ruminal contents after removal of the solid material. A Lovibond comparator was used for the *p*H determinations, sugar was estimated by Benedict's method and Rothera's test was used for acetone.

Observations

Following the second dose of glucose the cow was dull, rumination was in abeyance and there was inappetence. The faeces became yellowish-brown and soft in consistency, and a foul-smelling light-brown diarrhoea developed after the third dose which persisted until the fifth day. The animal showed a preference to eat straw on the fourth day but returned to normal health on the fifth day.

The observations made on the rumen contents are recorded in Table V. Phillipson (1946) quoted a rumen *p*H range of 5·8 to 7·1 and Dukes (1943), who stated that the reaction of rumen contents is slightly on the acid side of neutrality, gives a range of *p*H 6·0 to 7·8. It will be noted that some of the *p*H values obtained in this study appear higher than those quoted by Phillipson or Dukes. This is admitted and may be explained by the fact that occasionally

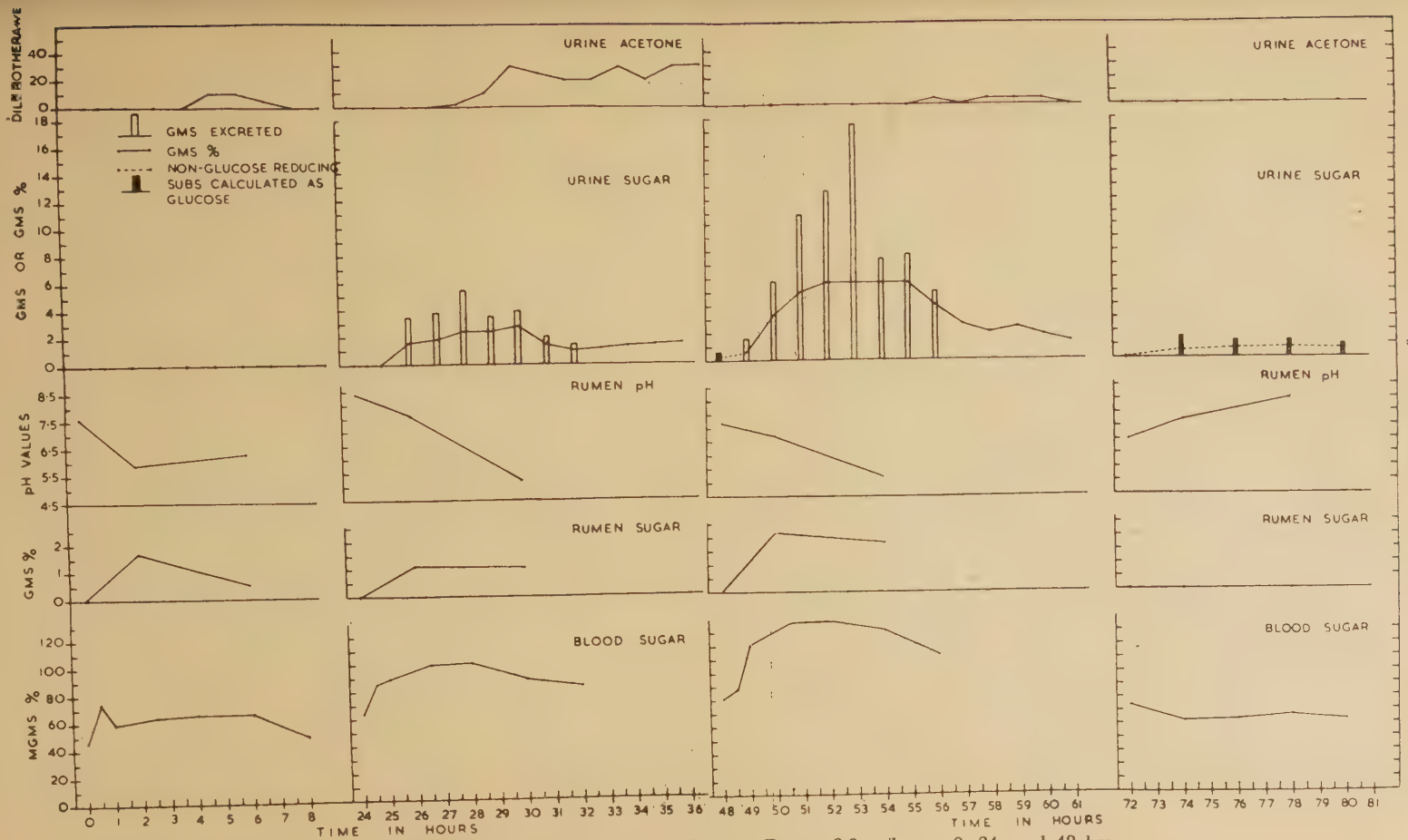

FIG. 4. Shorthorn Cow. Three-day experiment. Dose : 8·9 g./kg. at 0, 24, and 48 hrs.

samples of rumen contents were contaminated during collection by the highly alkaline saliva which flows in copious quantities on passing the stomach tube. It is likely, however, that the values recorded are not without significance. As observed by Phillipson and McAnally (1942), the administration of glucose caused a fall in the *p*H of the rumen contents and this was very marked following the second and third daily doses. On the first day the *p*H level tended to rise six hours after dosing, but it was still low 24 hours after the last dose had been given and did not return to normal until the following day. Acetone was not detected in the rumen samples obtained throughout the experiment. The rumen sugar concentration was related to the sugar content of the blood and urine in that it disappeared more slowly from the rumen in successive days.

TABLE V

THREE DAY EXPERIMENT : RUMEN CONTENTS.

Time	*Acetone*	pH. *Hrs. after dosing*			*Gm. % Sugar Hrs. after dosing*		
		0	*2*	*6*	*0*	*2*	*6*
1st day	−ve	7·6	5·9	6·3	—	1·7	0·56
2nd day	−ve	8·4	7·6	5·2	—	1·1	1·06
3rd day	−ve	7·2	6·7	5·2	—	2·2	1·8
4th day	−ve	*6·5	*7·2	*8·0	—	—	—
5th day	−ve	7·3	—	—	—	—	—

* 24, 26 and 30 hrs. respectively after dosing.

The blood sugar response increased progressively after each daily dose, so that while the level reached on the first day was similar to that observed in the previous experiments on this animal, on the second and third day it was greater and more prolonged, in fact the response to the third dose was not dissimilar to that observed in the calves. The rise was only transitory, however, and the level returned to normal 24 hours after the final dose (Table III). Glycosuria was related to the blood sugar response, being observed on the second day and most marked on the third.

It is interesting to note that the urine samples collected before dosing on the third day and all the samples obtained on the fourth day contained a reducing substance which was not glucose. This may have been due to liver embarrassment following large doses of sugar which resulted in the excretion of abnormal detoxication products.

TABLE III

THREE DAY EXPERIMENT : BLOOD SUGAR.

Time hours	*Predrenching level mg. %*	*Max. rise mg. %*	*Time of max. rise. Hours.*	*Time to return to normal. Hours.*
0	46	28	0·5	8
24	64	37	4·0	8++
48	71	57	4·0	8++
72	64	—	—	—
96	47	—	—	—

Acetone appeared in the urine following each dose of glucose. The excretion was most marked and persisted longest on the second day whilst on the third day it was very slight (Table IV).

Serum samples on the second and third days indicated an acetone level of 10 mg. per cent. All other samples were within the normal range.

TABLE IV

THREE DAY EXPERIMENT : URINE SAMPLES.

Time hrs.	*S.G. Range*	pH *Range*	*Acetone*	*Max. diln.*	*Sugar.*	*Total sugar excreted. Gm.*
0–24	1·028–1·057	5·5–7·5	+ve	1/10	−ve	—
24–48	1·002–1·039	5·5–6·0	+ve	1/30	+ve	23·4 *
48–72	1·003–1·047	5·5–6·0	+ve	1/5	+ve	68·15*
72–96	1·006–1·026	6·0–6·5	−ve	—	−ve	—

* Glucose excreted in the first 8 hours after dosing. Urine still glucose +ve 13 hours after dosing.

DISCUSSION

In comparing the results obtained in the young animals with those of the mature cattle, the striking difference is the much higher and more prolonged rise in blood glucose in the young animal compared with the very small and transient rise produced by a similar dose in the adult. The immature ruminant, certainly up to one year of age, appears to behave in a manner more closely resembling the single-stomached animal. This is in agreement with the observations of Bell and Jones (1945). Similarly, Allcroft and Strand (1933) found in sheep that the administration of high

carbohydrate meals to sheep previously fasted for 24 hours had a variable and small effect on the blood sugar as compared with the dog.

Phillipson and McAnally (1942) studying the fate of carbohydrates in the rumen of sheep reported that glucose is rapidly fermented to lactic acid. This is unstable and whilst some may be directly absorbed or pass on to the abomasum most is further fermented to volatile lower fatty acids. Phillipson (1947) observed that acetic acid is the predominant acid produced as a result of bacterial fermentation of carbohydrate in the rumen. Propionic and butyric acids are produced but in lesser quantities. Hence, in the adult bovine, most of the administered glucose is rapidly fermented to volatile lower fatty acids resulting in a negligible rise in blood sugar and a marked fall in rumen *p*H. This phenomenon is not so well established in the young animal, possibly because at that age the ruminal flora is incomplete, with the result that much more of the sugar is absorbed as glucose.

The clinical observations made in the three-day experiment indicate that large doses of glucose lead to anorexia, particularly for concentrated food, and that rumination is in abeyance. Diarrhoea also resulted. The fall in milk yield was very spectacular.

Another interesting feature is the absence of acetonuria following glucose administration in the young ruminant and its appearance in the adult. The failure of the young ruminant to excrete acetone, suggests that there may be a relationship between rumen function and acetone production.

The observation that following repeated glucose administration acetone and sugar were simultaneously excreted is a point of some interest. In the three-day experiment, however, acetone was not detected in the rumen contents. Whilst it is agreed that the amounts of acetone excreted in this experiment are relatively small compared with the values obtained in clinical cases of bovine ketosis, nevertheless, Rothera's Test is sufficiently sensisitive to detect the small quantities of acetone which presumably would be associated with these urinary concentrations, if they were formed in the rumen. This would therefore indicate that the acetone is formed from the volatile fatty acids after absorption and the liver is probably the site of formation.

The volatile lower fatty acids produced in the rumen of the adult animal are absorbed from the rumen, passing by way of the portal vein to the liver. When a large quantity of glucose is administered there will be a rapid absorption of the great excess of fatty acids formed in the rumen. It is reasonable to presume that some of the acetic acid molecules will combine to form acetoacetic acid and, in fact, this is one of the normal intermediate stages in metabolism via the tricarboxylic acid cycle. When large quantities of fatty acids accumulate in the liver then excess ketones will also be produced and lead to the excretion of acetone in the

urine. This is in accordance with the findings of Dye and McCandless (1948) who showed that most of the volatile fatty acids reaching the liver of the ruminant in the portal circulation are converted to ketone bodies and then utilised in the normal way presumably by way of the tricarboxylic acid cycle.

Observations during the three-day experiment on the concentration of sugar in the rumen, indicate that this remains higher for a longer period on each successive day, which suggests that possibly the rate of formation of the volatile fatty acids is reduced and, by the third day, they may be formed at a rate which the liver can safely deal with without any appreciable excess. Hence acetonuria is reduced. This suggestion is further supported by the rumen *p*H, which at the beginning of the fourth day was only 6.5 and rose throughout that day indicating that the formation of these volatile fatty acids was more prolonged during the third day than previously. Similarly in the other experiments the smaller doses of glucose resulted in a greater excretion of acetone. This may be due to the fact that the smaller dose was an optimum sugar concentration for rapid fermentation in the rumen resulting in the sudden influx of large quantities of lower fatty acids through the portal vein to the liver and hence the accumulation of excess ketone bodies.

In the Red Poll the smallest dose of 2·24 g./kg. resulted in a fall in blood sugar which may have been due to a sudden influx of lower fatty acids in the portal vein temporarily interfering with the liver's function of maintaining the blood sugar level. Hence, although the rumen may not be directly concerned in acetone production, it may be related to it in that conditions existing in the rumen may favour the production of large quantities of volatile fatty acids. The fact that in these experiments the calves did not excrete acetone, whereas the cows did, is an indication that this is so.

In the light of these observations one might consider the possible value of large doses of glucose by mouth in the treatment of bovine ketosis. A perusal of the literature shows that Shaw (1941), after pumping 6 lb. of glucose into the rumen of a cow with ketosis, found that the blood sugar rose from 15 mg. per cent. to 42 mg. per cent. 48 hours later. In normal bovines, Knodt (1941) found that after continuous glucose feeding at a level of 6 lb. per day for 10 days and 25 days respectively, the concentration of blood and urinary acetone bodies was about half that of the previously determined normal levels.

From the results obtained in these experiments, using normal animals and a relatively high dose of glucose, it is evident that only a small proportion of the administered sugar is absorbed as such. Sampson (1947) noted that the commonest post mortem finding in cases of bovine ketosis was "fatty changes within the liver" and there is reason to believe that liver glycogen stores are low (Shaw,

Hatziolos and Saarinen, 1948). If this is true, then liberal amounts of glucose should be beneficial. Nevertheless, in the ruminant, the production and absorption of large quantities of lower volatile fatty acids results in the excretion of ketones in normal animals, which would indicate that *large* doses of glucose by mouth, instead of being antiketogenic as in the single-stomached animal, are ketogenic in the ruminant and hence would be contraindicated in cases of clinical ketosis. As there is no appreciable blood sugar rise following glucose administered by the mouth in the adult bovine this route of administration is unsuitable as a liver function test in the ruminant.

SUMMARY

The young ruminant, at least up to 12 months of age, differs from the mature animal in its response to glucose administered by mouth, in that a much greater rise in blood sugar occurs than in the adult.

The administration of glucose to the adult animal in doses of 2.24 to 8.94 g./kg. resulted in the development of a temporary acetonuria after an interval of 1 to 5 hours: this did not occur in the young animal.

Following the glucose administration there was a marked fall in rumen *p*H.

No acetone could be detected in the rumen contents collected after glucose administration and during the period of acetonuria.

It is considered that these results indicate that in the mature ruminant glucose undergoes rapid fermentation to volatile lower fatty acids which are absorbed by the portal vein and normally undergo conversion to ketones within the liver prior to metabolism through the tricarboxylic acid cycle. When a sudden excess of these acids enter the liver ketones accumulate and are excreted in the urine. Hence the suggestion is advanced that the rumen is indirectly the source of the acetonuria due to the fermentation mechanisms and that the actual site of ketone production is probably the liver.

Whilst in the single-stomached animal glucose is antiketogenic when given by mouth, it appears that in the bovine, owing to its fermentation to volatile fatty acids, it may be ketogenic. This suggests that the treatment of ketosis with large doses of glucose may be contraindicated.

On account of the absence of any marked rise in blood sugar the oral administration of glucose will not provide a satisfactory hepatic function test in the bovine.

ACKNOWLEDGMENTS

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THE PUERPERIUM OF THE COW: A STUDY OF UTERINE MOTILITY*

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INTRODUCTION

Relatively few studies have been made of the physiology of the puerperium in animals and Williams (1943) states that no easily defensible standard for differentiating between a physiological and a pathological parturient cow has been established. The foetal membranes are normally expelled in from ½ to 8 hours after the birth of the calf and it is generally accepted that their retention in the uterus for 12 hours or longer is outside the normal physiological range. It is agreed by most authors that involution of the uterus is complete in 21 days after parturition and Hammond (1927) states that the cow usually ovulates at this time. Williams (1943), however, considers that the first heat period occurs 30 to 60 days after calving. Kennedy (1947) found degeneration and necrosis of the foetal membranes and of the cotyledons removed from normal cows at an average period 4¾ hours after parturition, while Gehring (1937) has described necrosis and oedema in 40 placentae expelled normally by clinically healthy cows.

The average duration of pregnancy in the bovine is 283 days; Dukes (1934) puts the range at 274 to 291 days. Parturition is associated with functional regression of the corpus luteum of pregnancy; although at the time of the act the yellow body is not reduced in size, it rapidly atrophies subsequent to parturition. Lowering of the progesterone plane and raising of the oestrogen level towards the end of pregnancy is said to be caused partly by functional changes in the placenta which, according to Kido (1937) and Gay, Jones and Hellman (1938), produces oestrogenic substances, progesterone and gonadotrophins in large quantities. These changes in the progesterone and oestrogen levels are associated with increasing sensitivity of the uterine muscle to the oxytocic factor of the posterior pituitory gland and the development of forceful uterine contractions. Murphy (1946) has described these contraction waves in the human being. He measured them, as a rule, by a tambour placed on the abdomen but sometimes by a balloon inserted into the uterus. Contractions commence during the last month of pregnancy and increase rapidly in strength and frequency to attain their maximum at the time of parturition. The sensitivity of the uterus to oxytocin rapidly diminishes during the first three days of the puerperium and after 10 to 15 days the uterus is completely quiescent.

* This article is based on part of the contents of a Thesis submitted for the degree of M.V.Sc. in the University of Liverpool.

Although the effect in vitro of various drugs on isolated strips of uterine muscle has long been recognised, the effect of the various hormone preparations in vivo has not been closely studied. Murphy (1946) has shown that in some cases the injection of pituitary extract in parturient women causes tetanic spasm of the uterine muscle. There have been many references in veterinary literature in recent years to clinical observations on the effect of stilboestrol on uterine contractility. Fisher, Maggon and Ranson (1938) have shown that damage to the neurohypophysis in cats is associated with inability to expel the foetuses.

The object of this study was to determine the changes which occur in the uterus of the cow post partum and to assess, if possible, the factors which favour retention of the foetal membranes and uterine infection. The paper also records observations made on uterine motility in the normal and abnormal puerperium, together with the effects of certain drugs and hormone preparations on uterine contractions.

EXPERIMENTAL

Observations were made during the parturient and post-parturient periods of 107 attested cows and heifers. With the exception of four, all belonged to three herds.

Herd A was self-contained and consisted of about 200 Ayrshires. Fertility, as calculated from the percentages of cows and heifers calving each year, was good and during the previous five years had varied between 87 and 100 per cent., the service-calving ratio varying annually between 1·24 and 2·03. During 1941 and 1942 animals infected with *Br. abortus* were eliminated from the herd by periodic blood testing. Following this, vaccination with McEwen's strain 45/20 was employed and since 1945, vaccination with strain 19 had been adopted. There had not been a case of brucella abortion in this herd since 1942.

Herd B consisted of about 120 Ayrshires. Fertility, while not as high as in Herd A, had been satisfactory and the milk yield from the herd was one of the highest in the area. *Br. abortus* infection had existed in this herd for many years. From 1945 onwards the young stock were vaccinated with strain 19 and during the period of three years preceding the commencement of this study animals had been re-vaccinated after their first calving. Occasional abortions due to *Br. abortus* occurred, the last positive one being in 1948.

Herd C belonged to the Veterinary Field Station of the University and consisted of some 20 tuberculin tested cattle of various breeds. As far as was known, *Br. abortus* infection had never occurred in this herd and vaccination of young stock with strain 19 was carried out.

Nature of the observations

Detailed clinical and haematological examinations were made

on the day of parturition and on the 4th, 8th and 15th days succeeding it. In addition the animals were examined on the 21st day for signs of the onset of heat. A swab from the cervix for bacteriological examination was taken on the day of labour and a blood sample for the determination of the *Br. abortus* agglutination titre on the 15th day. Many placentae were examined macroscopically and in some instances uterine cotyledons were removed for histological examination. In 50 animals, some of which were normal and others abnormal, kymographic tracings of the involuting uterus were made while in 22 others the effects of certain drugs on uterine contractions were recorded. The subsequent breeding history of the animals was noted.

Materials and methods

For measuring uterine contractions, a toy rubber balloon of about 30 ml. residual capacity was used. For introduction into the uterus it was attached to the anterior end of a Neilsen's uterine catheter (43 cm. long and 0·5 cm. external diameter). The posterior end of the catheter was connected by an 8 foot length of rubber pressure tubing with a mercury manometer. The manometer was in turn connected by a 4 foot length of tubing to a tambour which marked an electrically driven kymograph which rotated at a speed of 6 mm. per minute. Each observation occupied 1½ hours. After sterilisation the catheter carrying the balloon was introduced until the latter was situated in the posterior part of the recently gravid uterine horn adjacent to the posterior cotyledons. The stop-cock of the manometer was adjusted and by attaching a bicycle pump to the shorter length of rubber tubing, the balloon was distended. A single stroke of the pump served to do this. This distension of the balloon comprised simple filling of it with air without any stretching and its dimensions when so treated were four inches long and two inches broad at its greatest dimension. It was found that this simple filling of the balloon gave the most satisfactory results. The degree of pressure within the balloon was insufficient to cause a reading on the manometer and did not interfere with uterine contractions nor did it, by its bulk, cause uterine pain or irritation. In early experiments when the balloon was inflated to a diameter of four inches outside the animal's body, the pressure recorded on the manometer was 40 mm. Hg. Moreover, it was observed that when the balloon was distended to this degree within the uterus it was possible for the force exerted on it by uterine contractions to burst it. (It was difficult or even impossible to do this by squeezing it between the hands.) It is important to note that any sudden pressure or shock applied to the balloon, such as tapping, would cause the pointer of the manometer suddenly to swing up, but such a force would not sustain the reading and thus only a perpendicular line would be recorded on the drum. Such readings were produced by sudden movement of the animal, or by straining, bellowing or coughing. They varied in height from 2 mm. rises caused by body

movements, to rises of several centimetres caused by coughing. It is significant also that these elevations were highest in the atonic uterus in which no uterine contractions were being recorded, and lowest in those cases in which uterine contraction waves were strong and well-marked. Urination and defaecation produced small but distinct curves on the graph, resembling contraction waves but occurring spasmodically only. Respirations and ruminal contractions did not affect the recording. When recordings were being made during the first four or five hours post partum it was necessary to keep the balloon in the uterus by holding the catheter, otherwise the contractions would force it back through the cervix into the vagina. In later examinations, however, the cervix had generally contracted sufficiently to prevent expulsion of the balloon.

It was evident that the slow rhythmic waves recorded on the drum were uterine contractions. They could be felt by the hand introduced into the uterus; they occurred rhythmically and were slow and strong and they could not be ascribed to any other rhythmic involuntary movements of the animal body.

The kymographic tracings were taken in chronological order at various times without any selection of cases. In many instances in which the puerperium subsequently proved to be abnormal, the first tracings were taken immediately after parturition.

OBSERVATIONS

Animals With a Normal Puerperium

Forty-three recordings were made, 27 during the first 12 hours post partum and 16 at a later time. The general results of these observations are to be found in Table I, and are illustrated by Figs. 1, 2 and 3.

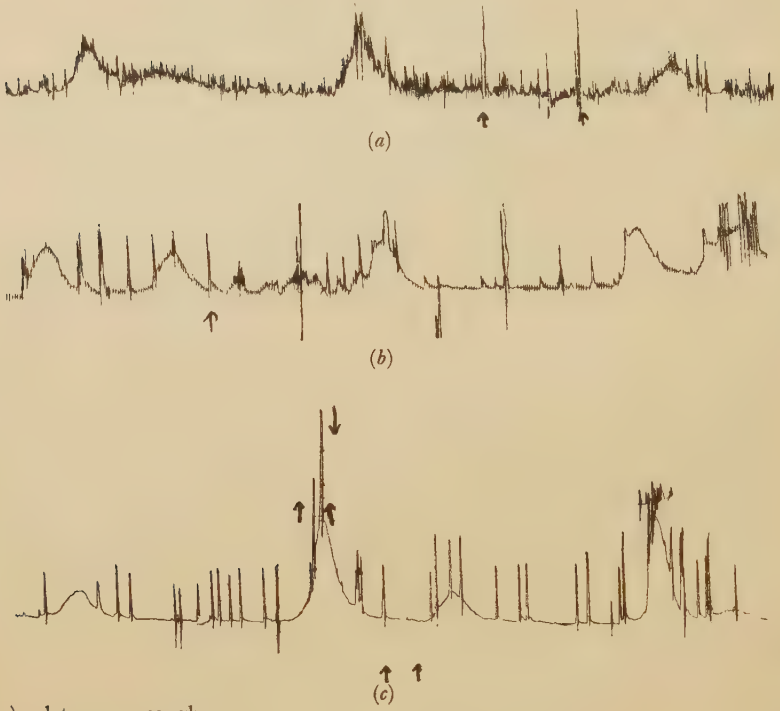
TABLE I

NORMAL UTERINE MOTILITY POST PARTUM

Hours post partum.	*No. of cases.*	*No. of contractions per hour*	*Strength (mm. Hg.)*	*Contraction time–mins.*	*Relaxation time–mins.*
0–12	27	13·3	20·2	0·7	1·07
More than 12	16	7·9	13·6	0·6	1·20

When recordings were made immediately after calving it was noted that forceful contraction waves occurred regularly about every three minutes. The average pressure exerted was 20 mm. Hg. although individual contractions in many cases greatly exceeded this. The onset of contraction was comparatively sudden and abrupt and the pressure rose to its full strength in a little over half a minute and remained at this level for 1 to 1½ minutes. At

FIG. 1. NORMAL COW.



(*a*) 1st ← = cough
2nd ← = straining
One hour before calf appeared. Note uterine contraction waves. Small numerous irregularities due to extreme restlessness.

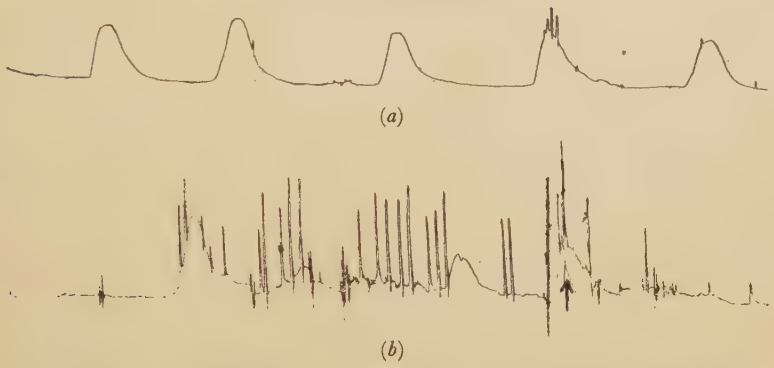
(*b*) Allantois appeared at vulva. Note restlessness slight. Small regular elevations due to laboured breathing, large sharp elevations due to forceful abdominal contractions.

(*c*) ← . . . ← = interval of ½ hour.
Immediately after calf was delivered. Uterine contractions strong. Straining pronounced but animal not restless.

the end of this time, pressure fell suddenly at first, and then gradually tailed off, making the end of the contraction period difficult to estimate exactly. As the period after parturition increased, uterine motility rapidly decreased although there were individual variations. At 12 hours post partum the average frequency was only one contraction every five minutes and the average strength had fallen to 18 mm. Hg; at 24 hours the frequency was about nine contractions per hour and the strength 16 mm. Hg. In three cases only were tracings taken on the second and third days after calving and in these the average frequency of contractions was four per hour and the strength 9 m.m. Hg. Voluntary straining occurred most frequently at the height of a contraction wave, that is, while the wave was passing over the balloon. Thus it would seem that the uterus is sensitive even to slight tension within it. In a few

instances recordings were being made while the placenta was being expelled. Expulsion occurred in stages coinciding with the contraction waves, and these were always accompanied by voluntary straining. Between waves the animal became quiet and often resumed cudding or eating. It was noticed that in those animals in which the placenta was quickly expelled, the sacro-sciatic ligaments

FIG. 2. NORMAL HEIFER.



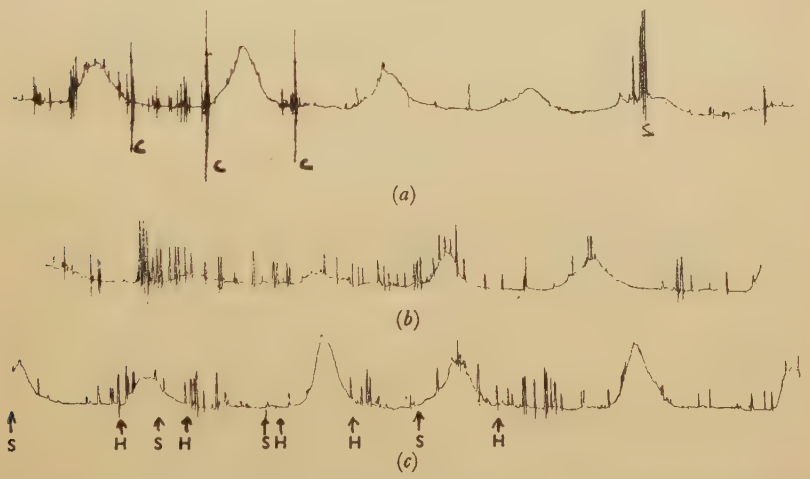
(*a*)

(*b*)

(*a*) One and a half hours after calving. Animal quiet. Note regular even strong uterine contraction waves.

(*b*) Increasing number of abdominal contractions as placenta loosens. Placenta finally passed at point marked.

FIG. 3. NORMAL COW.



(*a*)

(*b*)

(*c*)

(*a*) Ten minutes after calving. Animal still straining. Three sharp elevations marked '*c*' were due to coughs.

(*b*) Calf brought within sight of the mother.

(*c*) S = suckling
H = No suckling.
Calf was allowed to suckle dam periodically.

became taut in a matter of a few hours, whereas in those cases where contraction waves were absent (*vide infra*) the process was delayed. In all cases in which the placenta was expelled normally it was noted that the allantois chorion was inverted i.e. the allantoic surface was outermost. The placenta contained very little blood.

Direct observation of the separating placenta was made in a newly killed ewe. The uterus was exposed and incised and the lambs removed. Within five minutes involution of the uterus had commenced and by 20 minutes, it had contracted to about half its original size. Rhythmic waves of contraction passed from the apices of the cornua to the cervical extremity every three or four minutes. Occasionally a short wave of reverse contraction, commencing at the cervix and passing part of the way up the cornua, occurred. It was noticed that the waves of contraction caused the cotyledons to open up in a fan-like manner as they passed over them and it was found to be easier to detach the foetal from the maternal placenta at this time.

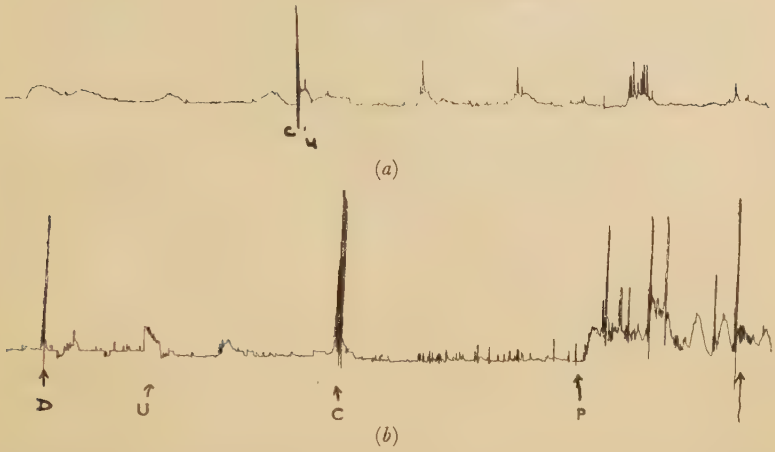
Having regard to the findings of Vinattieri, Hayward and Artioli (1945) on the influence of the suckling of the calf on the expulsion of the placenta in buffaloes, tracings were made with this point in mind. They were only attempted when recording was commenced immediately after calving and before the cow had had the opportunity of seeing or licking the calf. The first tracing was taken for about one hour and then the calf was taken to the cow and she was allowed to lick it. A little later the calf was allowed to suckle, and a tracing was taken for a further hour. No significant differences were observed between the records made before and after the calf was brought to the cow, nor did suckling affect the tracing (Fig. 3).

Animals With an Abnormal Puerperium

Seven animals are included in this group. In three of them, expulsion of the calf was unaided but they subsequently retained the foetal membranes; in two cases the birth of the calf was normal but the cows developed milk fever, while in the remaining two, the foetuses were aborted.

Retention. Tracings were commenced within three hours of the birth of the calf. Uterine contractions were greatly reduced or entirely absent, and in two of the three cases the tracing was almost a straight line. In one animal tracings were again made on the second and third days post partum. On the second day there was still complete absence of uterine movement but on the third day slight contractions had commenced. They comprised three waves of 6 mm. Hg. during the 1½ hours recording (Fig. 4). The membranes were subsequently expelled 4, 5 and 6 days later respectively. These retention cases were utilized later to observe the action of drugs on uterine contractions (*vide infra*).

FIG. 4. RETENTION OF PLACENTA.

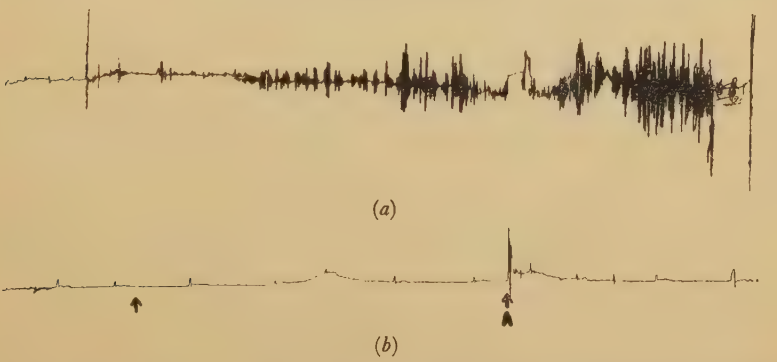


(*a*) Several hours after calving. Note the small feeble contraction waves.

(*b*) D = defaecation. C = cough.
U = urination. P = pituitary extract.
Reduced effect of intravenous injection of pituitary extract.

Milk fever. One animal was suffering from milk fever when first seen five hours after calving, and was bordering on coma when the tracing was taken. The other developed the condition eight hours after calving while the tracing was being taken and became recumbent and partially comatose. Both had expelled the foetal membranes before the onset of the disease. Uterine contractions were absent in both animals (Figs. 5 and 6). In both cases calcium borogluconate was given by intravenous injection. In the first, there was evidence that contractions began again thirty minutes

FIG. 5. MILK FEVER (CASE 1).

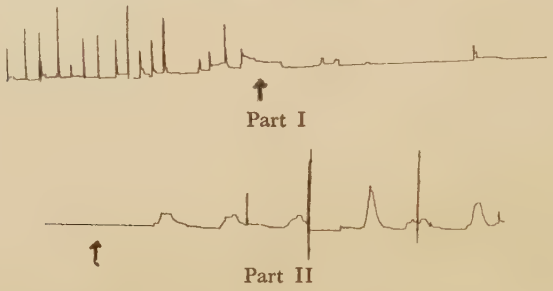


(*a*) Four to six hours after calving and shortly after cleansing. Note absence of uterine contraction waves and increasing restlessness till final collapse.

(*b*) First ↑ = Calcium borogluconate.
Absence of contraction waves while animal was clinically affected. Return to normal motility is gradual after calcium therapy.

after the injection and slowly regained their normal force. This return of uterine motility coincided with the cow's gradual return to consciousness. This statement is only partially confirmed by the kymographic record as the tracing ended soon after contractions had recommenced, but from this point the state of the uterus was assessed by rectal palpation. In the second case no contractions were observed within twenty minutes of the injection of calcium. Pituitary extract was then given and results are referred to later.

FIG. 6. MILK FEVER (CASE 2).



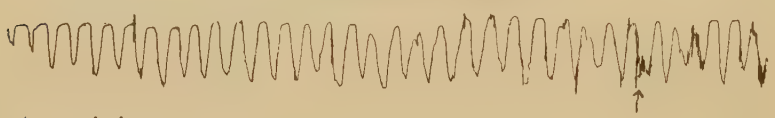
↑ Part I = calcium borogluconate

↑ Part II = pituitary extract.

Several hours after calving and shortly after cleansing. Note absence of motility but occasional straining. Calcium therapy produced no effect in 20 minutes. Pituitary extract provoked uterine motility. Note absence of characteristic tetanic response after second injection.

Abortion. Both cases occurred during the fourth month of gestation and were unassociated with *Br. abortus* infection. In one a tracing was obtained between the third and fourth hours after abortion and in the other there was a delay of 38 hours. A striking feature of both was the high frequency of contractions of moderate force. Even in the case in which the recording was delayed, contractions occurred every two minutes (Fig. 7).

FIG. 7. ABORTION AT 7½ MONTHS.



↑ = pituitary extract.

Very frequent waves of contraction without full relaxation. Pituitary extract produced no obvious response.

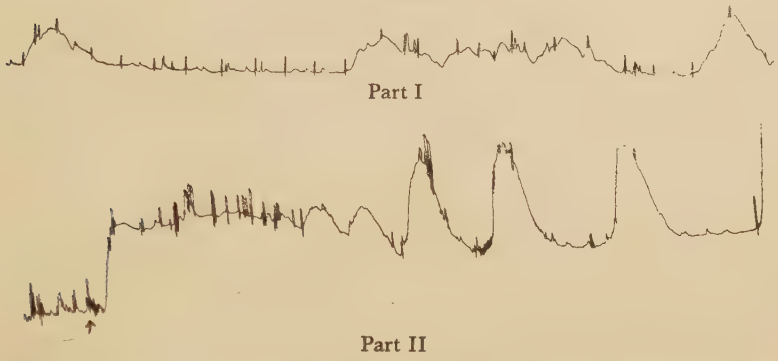
The Effect of Drugs on Uterine Contractions in the Normal and Abnormal Uterus

Pituitary Extract

The effect of pituitary extract ("Infundin"—Burroughs Wellcome & Co.) given by intravenous injection in a dosage of 30 I.U. was observed in seven normal and six abnormal cases.

Normal. The results are summarized in Table II. From this it will be seen that the effect of the drug in general was to increase considerably both the force and frequency of contractions although there were variations (Fig. 8). This response was constant although

Fig. 8. Normal Cow.



Twelve hours after calving. Part I shows the normal uterine contraction waves and Part II the effect of pituitary extract.

the time of the injection after parturition varied from $1\frac{3}{4}$ to 21 hours. The response was greatest when the drug was given soon after parturition. The onset of the drug's action was almost immediate (within 20 seconds of injection) and comprised a tetanic spasm of the uterine muscle lasting for periods of from four to nineteen minutes. The duration of the spasm was not related to the interval between parturition and injection. After the spasm had passed, contractions became rhythmic. The effect of the drug remained constant for about one hour, after which it gradually subsided and was lost by the end of the second hour.

Table II

Effect of Pituitary Extract on Uterine Motility

Case No.	*Hours Post partum*	*Before Pituitary Extract*		*After Pituitary Extract**	
		Frequency No. per hour	*Strength mm. Hg.*	*Frequency No. per hour*	*Strength mm. Hg.*
C 36321	$1\frac{3}{4}$	22·2	25·2	17·1	44·6
P 290	$3\frac{3}{4}$	10·5	39·2	17·4	55·2
H 144	4	6·3	8	22·2	18·4
L.S.	$4\frac{1}{2}$	9·2	41	12·5	38·8
407	5	15·2	14·4	27·3	37·0
L.R.P.	13	9·5	15·6	18·5	49·0
P 234	21	8·1	15·0	15·8	25·0

* 30 I.U. Pituitary Extract intravenously.

Abnormal. Pituitary extract was given on two occasions to two cases of retention of the placenta. In each case while the drug influenced uterine contractions, the effect was much less, both as regards strength and frequency, than in a normal case (Fig. 4).

In one case of milk fever in which uterine contractions were absent pituitary extract was given twenty minutes after treatment with calcium. Uterine motility was uninfluenced until twenty minutes later by which time the animal was rapidly recovering from the milk fever and it was considered that the effect was due to this, rather than to pituitary extract. In both cases of abortion, pituitary extract produced a greater uterine response than in the normal animal.

Stilboestrol

The effect of the parenteral administration of 30 mg. of stilboestrol dipropionate in oil (May and Baker) was studied in six normal cases and in one case of retention. In three animals the drug was given intravenously and in four intramuscularly.

In all normal cases administration was on the day of calving. No change in uterine motility was noted during the period of the tracing (about one hour), nor when tracings were again taken six to eight hours later or on the following day.

An intravenous injection in a case of retention of five days duration also produced no observable effect.

Stilboestrol and Pituitary Extract

In two normal cases referred to previously in which 30 I.U. of pituitary extract were injected intravenously, 30 mg. stilboestrol dipropionate was injected intravenously after 1½ and 2 hours respectively. No change in uterine motility was observed. When, however, a second injection of pituitary extract was made half an hour after the stilboestrol, the response was most marked, being about twice as forceful as that following the original injection (Fig. 9).

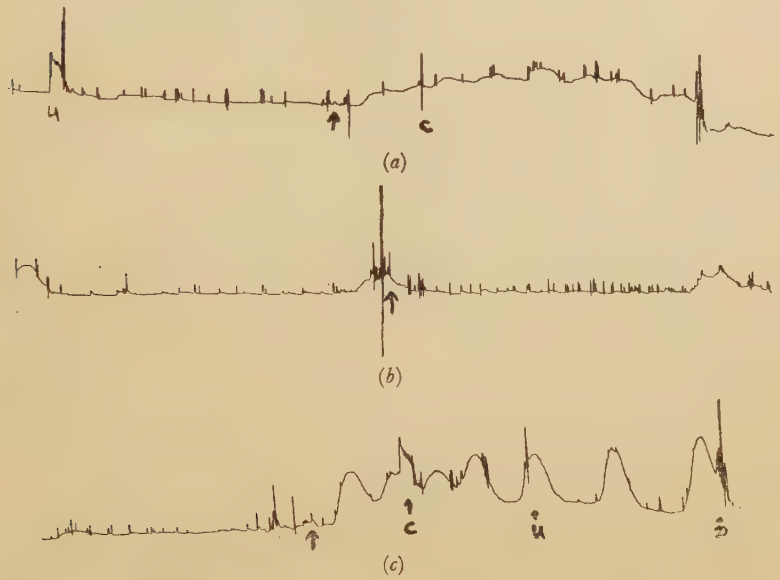
The effect of the two drugs was also studied in one case of retention of the foetal membranes in which there was atony of the uterus. The first tracing was commenced 16 hours after calving and at this time the intramuscular injection of pituitary extract had very little effect. After half an hour, 30 mg. stilboestrol was injected intravenously and again there was no appreciable response. After a further half hour's delay a third dose of pituitary extract was given intramuscularly and although there was some response, it was still below normal. At 41 hours after calving the response to an intramuscular injection of pituitary extract was greater than previously seen and at 71 hours it was greater still.

Progesterone

In three normal cases 20 mg. progesterone (Burroughs Wellcome and Co.) was injected intravenously on the day of calving. No appreciable effect on uterine motility was observed.

Thirty minutes later pituitary extract was administered intravenously and the response was significantly less than normal (Fig. 10).

FIG. 9. NORMAL COW.

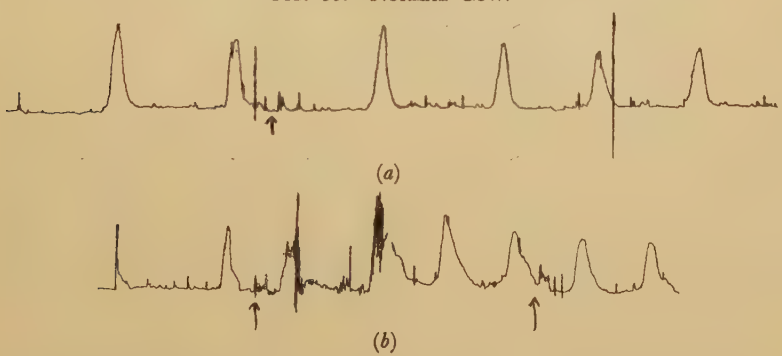


(*a*) ↑ = pituitary extract.
One hour after calving showing effect of pituitary extract.

(*b*) ↑ = stilboestrol.
After reaction to pituitary extract had ceased, no response to stilboestrol.

(*c*) ↑ = pituitary extract.
One half hour later shows the increased effect of pituitary extract.

FIG. 10. NORMAL COW.



(*a*) ↑ = progesterone.
Twelve hours after calving. Strong uterine contractions uninfluenced by progesterone.

(*b*) ↑ . . . ↑ = expulsion of placenta.
One half hour later ; contraction waves uninfluenced by pituitary extract or only very mildly so, thus demonstrating the desensitising effect of progesterone.

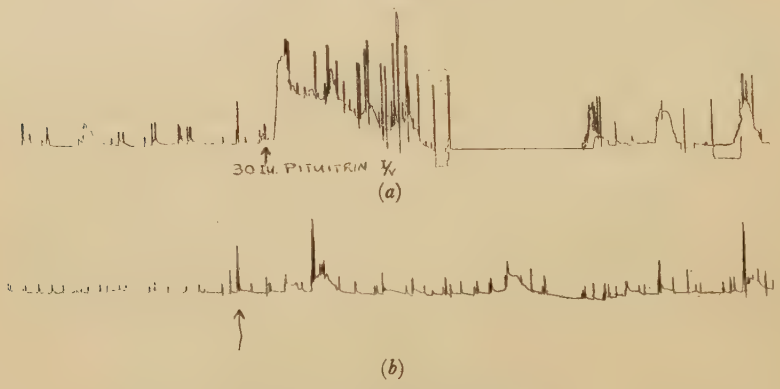
Testosterone

The effect of 200 mg. testerone propionate (Organon Laboratories) was observed in two normal cases. In one the whole dose was injected intravenously and in the other half was given intravenously and half intramuscularly. The drug had no effect on motility and did not modify the response to pituitary extract.

Ergometrine

Ergometrine malleate (May and Baker) was used on the day of parturition in four cases; three normal and one of retention. In the first normal animal 3 mg. were injected intravenously. The effect, which was delayed was only slight (Fig. 11). In the second 6 mg. were given by the same route. After a delay of five minutes the response was about the same as that obtained with the smaller dose. In the third case 12 mg. were given with similar results. In the case of retention with uterine inertia 6 mg. provoked slight contractions.

FIG. 11. NORMAL COW.



About 36 hours after calving.
(*a*) Tetanic response to pituitrin.
(*b*) No response to ergometrine.

Acetylcholine

Three c.c. of 0·1 per cent. solution of acetylcholine were injected intravenously in a normal animal, 41 hours after calving. A strong uterine contraction wave almost immediately resulted, lasting between one and two minutes, after which contractions returned to normal.

Procaine (Epidural anaesthesia)

Six cc. of a 2·25 per cent. solution of procaine hydrochloride were injected into the epidural cavity at the first intercoccygeal space in a normal cow on the day of calving. Uterine motility was unaltered but it was noticed that the animal, which had been restless, became tranquil with the onset of anaesthesia. A similar result was obtained in a case of abortion.

DISCUSSION

From the observations made it is possible to propound a theory of the separation of and expulsion of the foetal membranes and, indeed, of the act of parturition itself. Sensitivity of the uterus to oxytoxin increases towards the end of pregnancy. This must be due in part to the functional degeneration of the corpus luteum and to a reduction in the progesterone level in the blood for it has been shown that progesterone reduces the sensitivity of the uterus, and in part to an increase in oestrogen level for it has also been demonstrated that stilboestrol sensitises the uterus to pituitrin. During labour, blood is squeezed from the placenta into the foetus thus emptying the placental villi and assisting in dehiscence. Immediately the calf is born the uterus shortens in length and circumference, and this, together with strong waves of contraction passing from the tip of the cornu to the cervix, completes the separation of the placenta which is then pushed out, starting at the apex of the horn turned inside out. The animal assists by straining which occurs most often during the contraction wave in the uterus. It would seem that straining is initiated by sensations from the uterus, possibly those of tension or pressure on an ischaemic area of uterine muscle. This theory of separation and expulsion of the membranes is supported by observations made in cases showing disturbances in the normal pattern of events. When the placenta is retained uterine motility is greatly reduced or even absent. In cases of milk fever also, uterine activity is in abeyance but it is interesting to note in the latter connection that, with the recovery of consciousness following appropriate treatment, uterine activity is immediately restored. The view that nervous impulses initiated reflexly or even psychologically as by the licking of, or the suckling by the calf, aid placental separation could not be demonstrated by an increase in the force or frequency of uterine contraction waves.

It is possible that the cause of the uterine hypersensitivity in abortion at the fourth month of pregnancy is an overproduction of oestrogens together with a deficiency of progesterone, for the uterus is very motile, involutes very rapidly and the onset of heat is abnormally early.

The work under review confirms the finding in the human subject that the intravenous injection of pituitary extract provokes a tetanic contraction of the uterus and also that if administration is subcutaneous the tetanic phase is much reduced but the action of the drug is prolonged. Uterine sensitivity to pituitary extract gradually diminishes post partum, but is still apparent at the third day. It is widely thought that stilboestrol stimulates uterine contractions but these observations have shown that it does not. Its action is to sensitise the uterus to pituitary extract and if the latter is given after the former, there is a great increase in response. It is to be noted that while the induction of posterior epidural anaesthesia eliminates straining it does not affect uterine motility.

Retention of the placenta is probably not caused primarily by bacterial infection at parturition. It is more probable that both retention of the placenta and post parturient infection are brought about by delayed uterine involution.

SUMMARY

Clinical observations were made during the puerperium of 107 attested cows and heifers. The motility of the involuting uterus was studied in 50 cases and the action of drugs on uterine contractions in a further 22.

The normal patterns of uterine motility during the first 12 hours after expulsion of the foetus and during the later periods are described.

In seven cases in which the puerperium was abnormal, uterine contractions are compared with the normal pattern.

No significant changes in uterine contractions was observed during periods when the cow was allowed to lick the calf or during suckling.

The actions of pituitary extract, stilboestrol and other drugs on uterine motility during the normal and abnormal puerperium are described.

Evidence is advanced to support the view that retention of the foetal membranes is associated with reduced uterine motility within 24 hours after calving.

ACKNOWLEDGMENTS

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THE INEFFECTIVENESS OF MEPACRINE IN THE TREATMENT OF COCCIDIOSIS OF CHICKENS, MICE AND SHEEP

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INTRODUCTION

For some years there have been reports in the Continental veterinary literature of the successful use of Mepacrine (Quinacrine or Atebrin) in the treatment of coccidiosis of cattle, rabbits and cats (Perrin, 1942; Brumpt, 1943 a & b). These articles as well as a note on its use in England in cattle (Edgson, 1948) were unconvincing, as no controls were kept to observe the progress of the untreated infection.

In the evaluation of the treatment of such a disease as coccidiosis this is a most important point, as unless there is an acute, explosive and severe type of outbreak affecting a large number of animals, it is very difficult to decide if recovery has been natural or has been due to the drug used for treatment. Furthermore the faecal oocyst counts are also liable to be misleading unless performed on a large number of animals, as there is often great day to day variations in the oocyst output. It is, for instance, easily possible to take samples on suitable days rather late in the course of heavy but limited infections with coccidia and to demonstrate a very marked drop in oocyst production from the previous day. If the details of the cases recorded by Edgson are examined it is clear that they were only slight cases, as he states "none exhibited an acute enteritis" and the calves probably made spontaneous recoveries; a two-year-old bull, however, showed rather more marked symptoms—at the end of the third day of treatment the blood in the bull's faeces was more than at the commencement of treatment, and blood "was still present in similar amounts" by the fifth day. Although this was the most severe case in the series, in my opinion it was relatively mild—in fact, Edgson himself says "the animal appeared well". It failed to respond to treatment, at all events, as compared with the recovery which can be expected from the use of sulphone (Steward, 1947) or sulphamezathine.

Some years ago Mepacrine was tested in experimental duodenal and caecal coccidiosis in chickens and also in an explosive natural outbreak in sheep—in all instances without evidence of effect. It was therefore surprising when a note appeared on the value of Mepacrine in experimental caecal coccidiosis of fowls (Wilson, 1949). Examination of his results did not show very marked evidence of therapeutic effect as 14 out of 60 (23 per cent.) birds died when treated with Mepacrine from 24 hours after infection, as

compared with 49 out of 80 (61 per cent.) untreated birds. The fact that mortality in the untreated birds did not exceed 61 per cent. suggests that the infective dose was not very high. Later results are published (Wilson, 1950) which show that even at double the dosage previously used " the earlier promise of the drug was not maintained ". In the latter note Wilson records that the mortality in two groups of chickens treated for three and seven days prior to and until five days after exposure to infection was 64 and 57 per cent. as compared with 92 per cent. in the untreated. The death rate in a group treated with sulphamezathine from 72 hours after exposure to infection was only 4 per cent.

Unsworth (1949) found that the drug was ineffective in rabbits.

RESULTS

Experimental Coccidiosis

In view of the unfavourable results obtained by myself in the treatment of natural coccidiosis in sheep and experimental coccidiosis in chickens compared to those recorded by Perrin and Brumpt *et al.*, it was considered desirable to make further tests of Mepacrine against experimental coccidiosis in mammals. For this purpose mice were chosen, as laboratory bred mice are not often naturally infected with coccidia, whereas rabbits are difficult to maintain and raise free from this infection. Furthermore, the life cycles of the coccidia of rabbits are protracted, whereas those of the mouse are short. In addition, rabbits are poor subjects for treatment with the sulphonamides, which would be used for the control treatments of coccidiosis, for they are rapidly broken down into inert substances.

In chickens, Mepacrine was tested against coccidiosis. In the duodenal type due to *Eimeria acervulina* the effect was estimated by changes in the oocyst counts, whereas with the caecal infection due to *E. tenella* the interpretation was based upon the post-mortem lesions and the death rate. With both infections different routines of dosage have been adopted in order to detect both prophylactic and therapeutic activity.

In all cases dosing has been twice daily and the time of starting has been indicated in the tables; thus the day on which treatment is started is indicated by a minus sign if it was prior to infection, and a and b represent morning and evening dosings. The doses used were close to the maximum tolerated and in some cases bordered on the toxic.

Prophylactic Effect

Mouse coccidiosis

When treated with Mepacrine from 3 hours prior to infection there appeared to have been no effect (Test III) but when treatment started 19 hours before infection and lasted for four days there was a slight protection. (Table I.)

TABLE I

THE PROPHYLACTIC EFFECT OF MEPACRINE ON INTESTINAL COCCIDIOSIS IN MICE.

Test No.	*Compound*	*Dose per 20 gm. mouse*	*No. of doses*	*Days of dosing*	*No. of mice*	*Av. change in weight 7th day*	*Mice negative*		*Av. coccidia count pl.*		*Deaths*	*Results*
							6th	*7th*	*6th*	*7th*		
III.	Untreated	—	—	—	8	0	0:8	0:8	440	100	0	—
150,000 oocysts	Sulphamezathine	15 mg.	6	0 to + 2	8	+1 gm.	2:8	1:8	55	35	0	*Incomplete effect at this stage of infection.
	Mepacrine	3 mg.	6	+0 to +2	8	−3 gm.	0:8	0:7	520	115	1	Inactive.
	Mepacrine	5 mg.	6	+0 to +2 (3 hrs. ante infection)	8	−4gm.	0:7	0:7	280	100	0	Slight activity.
V.	Untreated	—	—	—	10	−1 gm.	2:10	0:8	95	105	0	—
100,000 oocysts	Sulphamezathine	12 mg.	7	72 hrs. P.I.	10	−2 gm.	9:10	6:10	0	0	0	Control (therapeutic — not prophylactic).
	Mepacrine	3 mg.	7	−1b to+2b	12	−1 gm.	0:9	2:7	105	40	2	Possible slight effect.
	Mepacrine	5 mg.	6	−1b to +2a (19 hrs. ante infection)	12	−3 gm.	0:9	3:5	300	10	1	Possible slight effect.
VII.	Untreated	—	—	—	10	+1 gm.	1:10	—	76	—	0	—
100,000 oocysts	Sulphamezathine	15 mg.	8	+2b to 6a	30	+1·85 gm.	2:5	—	2	—	2	Active control (therapeutic not prophylactic).
	Mepacrine	3 mg.	7	−1a to +2a	30	−2·35 gm.	1:22	—	88	—	4	Inactive.

P.I. = Post Infection.

See Table II for notes.

TABLE II

THE EFFECT OF MEPACRINE IN EIMERIA ACERVULINA INFECTION IN CHICKENS.

Infective Dose Test No.	*Compound*	*Dose per 50 gm.* †	*No. of Doses*	*Time 1st dose P.I.*	*No. of chicks*	*Deaths*	*Day of Death*	Survivors			*Effect of compound*	*Birds negative*
								No.	*Av. change weight 5th day*	*Intensity of infection*		
Test 25 150,000 oocysts	Uninfected ...	—	—	—	4	0	—	4	+8 gm.	—	—	—
	Untreated ...	—	—	—	4	0	—	4	+8 gm.	450 pl.	—	0:4
	Sulpha-mezathine	12·5 mg.	11	48 hrs.	4	1	3.	3	—	0	Active control	3:3
	Mepacrine ...	5 mg.	8	48 hrs.	6	4	2, 2, 3, 4.	2	+ 7gm.	200 pl.	Some activity	1:2 (4th day)
	Mepacrine ...	7·5 mg.	8	48 hrs.	5	3	3, 5, 6.	2	+5 gm.	200 pl.	Some activity	1:2 (5th day)
Test 38 150,000 oocysts	Uninfected ...	—	—	—	6	4	3, 3, 3, 5.	2	+2 gm.	—	—	—
	Untreated ...	—	—	—	6	2	4, 5.	4	+7 gm.	300 pl.	—	0:4
	Sulpha-mezathine	12·5 mg.	7	48 hrs.	6	4	3, 4, 5, 6.	2	+5 gm.	0	Active control	3:3
	Mepacrine ...	8 mg.	7	48 hrs.	8	4	3, 4, 5, 6.	4	+5 gm.	300 pl.	Inactive	0:5
Test 165 120,000 oocysts	Untreated ...	—	—	—	19	15	3, 4, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6, 6, 6, 6.	4	+2 gm.	210 pl.	—	0:16
	Sulpha-mezathine	6 mg.	8	48 hrs.	19	5	—	14	+13 gm.	0	Active control	14:14
	Mepacrine ... (prophylactic)	4 mg.	6	0–2b	19	13	4, 5, 5, 5, 5, 5, 6, 6, 6, 2, 2.	6	+5 gm.	150 pl	Inactive (prophylaxis and early chemotherapy)	0:17

Test 166 100,000 oocysts	Uninfected ...	—	—	—	9	0	—	—	+9 gm.	—	—	—	
	Untreated ...	—	—	—	19	8	4, 5, 5, 5, 6, 6, 6, 6.	11	+<1 gm.	155 pl.	—	0:16	
	Sulpha-mezathine	6 mg.	9	48 hrs.	20	1	4.	19	+14 gm.	0	Active control	19:19	
	Mepacrine ... (prophylactic)	4 mg.	6	0–2b	19	6	4, 4, 5, 5, 5, 6.	13	+3 gm.	150 pl.	Inactive (prophylactic)	0:16	
Test 175 130,000 oocysts	Untreated ...	—	—	—	8	0	—	8	+18 gm.	115 pl.	—	0:8	
	Sulpha-mezathine	6 mg.	5	48 hrs.	8	0	—	8	+16 gm.	0	Active control	8:8	
	Mepacrine ...	6 mg.	5	48 hrs.	60	5	5, 5, 5, 5, 5.	55	+7 gm.	85 pl.	Inactive	*4th* 31:60	*5th* 1:55

NOTE : Interpretation is based partly on the number of birds negative and partly on oocyst counts as compared with the controls.

* Time of Dosing *a* represents morning and *b* evening dose thus—1b means dosed evening before infection—1a from morning of day before infection, 2b = evening of 2nd day of infection.

ABBREVIATIONS : P.I. = post infection, pl. = per "lane" of coverslip (for total coverslip count ×8).
† Treatment given twice daily.

TABLE III
THE EFFECT OF MEPACRINE IN EIMERIA TENELLA INFECTION IN CHICKENS.

Infective Dose and Test No.	*Compound*	*Dose per 50 gm.*	*No. of Doses*	*Time 1st Dose P.I.*	*No. of birds*	*Deaths before 5th day*	*Deaths 5th-7th day*	*Day of Death*	*Birds Negative*	*Average lesions*	*Effect of Compound*
Test 167	Untreated ...	—	—	—	15	0	10	6, 5, 5, 5, 5, 5, 5, 6, 6, 7.	0:15	3·5 +	—
25,000 oocysts	Sulpha-mezathine	20 mg.	8	78 hrs.	14	0	1	5.	0:14	1·8 +	Active (control).
	Mepacrine ...	5 mg.	8	78 hrs.	14	0	7	5, 5, 5, 5, 5, 6, 6.	0:14	>+++	Inactive.
	Mepacrine (prophylactic)	5 mg.	7	3 hrs. prior to infection	13	0	8	5, 6, 6, 6, 6, 6, 7.	0:13	3·5 +	Inactive.
Test 168	Untreated ...	—	—	—	13	0	8	5, 5, 5, 6, 6, 6, 7, 7	0:11	>+++	
25,000 oocysts	Sulpha-mezathine	20 mg.	10 (2b–7a)	54 hrs.	14	0	0	—	5:14	1·5 +	Active (incompletely).
	Mepacrine ...	5 mg.	10 (2b–7a)	54 hrs.	14	4	8	3, 3, 4, 4, 5, 5, 5, 5, 5, 5, 5, 7.	0:10	<++++	Inactive.
	Mepacrine ... (prophylactic)	5 mg.	8 (–1b–3a)	20 hrs. prior to infection	14	0	3	6, 6, 7.	2:14	++	Slight activity.
Test 172 26,000 oocysts	Untreated ...	—	—	—	25	0	20	5, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6.	0:20	+++	—
	Sulpha-mezathine	25 mg.	10	30 hrs.	23	0	0	—	0:12	+	Active (control).
	Mepacrine ...	5 mg.	12 (1a–4b)	24 hrs. prior to infection	25	0	19	5, 5, 5, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 7, 7, 7.	1:25	+++	Inactive.
	Mepacrine ...	5 mg.	10	30 hrs.	25	1	20	5, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6, 6.	0:25	+++	Inactive.

FOOTNOTE : Interpretation is based on pathological change found at Post-mortem and the following definitions are used :—
(1·5) ++ may be regarded as slight disease.
+ may be regarded as very slight evidence of disease.
(2.5) +++ and over is to be regarded as severe disease.

Eimeria acervulina

Against this species when dosing started a few hours before infection and lasted three days there appeared to be no effect, as no birds were negative and the intensity of the infection was not reduced. (Table II.)

Eimeria tenella

In two tests against this coccidium there was no prophylactic effect from Mepacrine. In a third test (168) in which Mepacrine was started 20 hours prior to infection, there appeared to be slight activity since the average severity of the lesions was reduced and two out of fourteen birds were free from lesions, but in a later test when treatment started 24 hours before infection there was no effect. (Table III.)

Therapeutic Effect

Mouse coccidiosis

When dosage rates of 3 or 5 mg. per 20 g. mouse commenced 72 hours after exposure to infection, there appeared to have been some slight activity. Thus, on the sixth day of infection there were 6/22 mice negative on the 3 mg. regime and 1/20 negative on the 5 mg. rate, as compared with 2/27 in the untreated group. The figures for the seventh day were 4/16 and 3/12 for the 3 and 5 mg. groups and 0/18 for the untreated. There was a slight reduction in the oocyst counts on the sixth day for the 5 mg. groups and a rather greater reduction for the 3 mg. group. The oocyst counts were lower still, where recorded, for the seventh day. The 3 mg. dosage regime was the more effective, but the 5 mg. dosage course was toxic, though not fatally so. On sulphamezathine 24 out of 28 mice were negative. (Table IV.)

Eimeria acervulina

There was a slight but variable effect with high dose courses of Mepacrine starting at 48 hours after exposure to infection.

Eimeria tenella

Whether treatment started at 30, 54 or 78 hours after exposure to infection there appeared to be no therapeutic effect. If judged by mortality rather than average degree of lesions the proportions of deaths known to be due to coccidiosis among the treated was 35* out of 53 birds as compared with 38 out of 56 in the untreated. The difference appears insignificant. On the sulphamezathine controls the deaths numbered 1 out of 51 birds.

In general there appears to be an occasional but variable and unpredictable response to Mepacrine in the treatment or prophylaxis of experimental mouse coccidiosis. In the chicken it is probable that where there is a high infective dose of a very pathogenic coccidium, such as *E. tenella*, Mepacrine is without value in the treatment of the infection.

* Three deaths before the fifth day are excluded from this figure as no lesions are recorded to indicate the birds died from coccidiosis.

TABLE IV

THE THERAPEUTIC EFFECT OF MEPACRINE ON INTESTINAL COCCIDIOSIS IN MICE.

Test No.	*Compound*	*Dosage level/ 20 gm.*	*No. of doses*	*Time 1st dose P.I.*	*No. of mice*	*Average change in weight*	*Mice negative*		*Av. coccidial count*		*Deaths*	*Results*
							6th	*7th*	*6th*	*7th*		
I.	Untreated	—	—	—	5	−2 gm.	0:4	—	180	—	1	—
90,000 oocysts	Sulphamezathine	9 mg.	7	72 hrs.	5	−½ gm.	5:5	—	0	—	0	Active (control).
	Mepacrine	3 mg.	7	72 hrs.	5	−3 gm.	1:5	—	25	—	0	Partial activity.
II.	Untreated	—	—	—	4	−6 gm.	0:4	—	190	—	0	—
90,000 oocysts	Sulphamezathine	7·5 mg.	3	72 hrs.	4	−3 gm.	2:4	—	18	—	0	Active (control).
	Mepacrine	5 mg.	3	72 hrs.	4	−4 gm.	0:3	—	80	—	1	Some activity.
III.	Untreated	—	—	—	10	+1 gm.	0:9	0:10	180	110	0	—
120,000 oocysts	Sulphamezathine	12 mg.	7	72 hrs.	10	+1½ gm.	8:9	9:9	4	0	0	Active.
	Mepacrine	3 mg.	7	72 hrs.	10	−½ gm.	1:9	0:9	150	100	0	Inactive.
	Mepacrine	5 mg.	6	72 hrs.	10	−1 gm.	1:8	0:6	180	130	3	Inactive.
IV.	Untreated	—	—	—	10	−1 gm.	2:10	0:8	95	105	0	—
100,000 oocysts	Sulphamezathine	12 mg.	7	72 hrs	10	−2 gm.	9:10	6:6	0	0	0	Active (control).
	Mepacrine	3 mg.	7	72 hrs.	11	−1 gm.	4:8	4:7	105	65	1	Some activity.
	Mepacrine	5 mg.	7	72 hrs.	11	−4 gm.	0:9	3:6	430	70	0	Some activity.
VII.	Untreated	—	—	—	10	+1 gm.	1:10	—	76	—	0	—
100,000 oocysts	Sulphamezathine	15 mg.	8	50 hrs.	8	+1·85 gm.	2:5	—	2	—	2	Active control.
	Mepacrine ... ... (therapcutic).	5 mg.	8	50 hrs.	30	−2·6 gm.	2:21	—	100	—	9	Inactive and toxic.

Natural Coccidiosis

The only opportunity which arose to test Mepacrine in a natural outbreak of coccidiosis in ruminants occurred in a very acute, explosive, outbreak affecting 145 sheep. The outbreak was detected early but a large number of sheep were affected with dysentery and tenesmus. The flock was divided into 13 groups of from ten to thirteen sheep and all except one were treated once daily with sulphonamides or sulphones, and one group twice daily with Mepacrine. One group acting as "untreated" controls received chlorodyne and chalk, a traditional but ineffective remedy. An understanding existed with the owner that as soon as it was clear that the treatment of any group was unsuccessful it would be put on to a successful treatment, and this was fulfilled in relation to the untreated, Mepacrine, phthalyl sulphathiazole, and sulphanilamide groups after three days' treatment, as by this time other treated groups showed great clinical improvement. It is not proposed to record the sulphonamide results here but only to quote one or two as examples.

It was only possible to sample half the flock each day but by the third or fourth days after treatment the group average and most individual oocyst counts fell in all but three groups. The unimproved groups were the untreated, the Mepacrine treated, and phthalyl sulphathiazole treated. The sulphanilamide group was also doing very badly but this was on account of toxicity, as sulphanilamide is not well tolerated by sheep. These four treatments were replaced from the fourth day by the more successful treatments, e.g. sulphamezathine and a sulphone. The sheep were weighed on the first, sixth and fifteenth days. It is possible that the sixth day weights in these four groups may have been just influenced by the later successful treatment. (Table V.)

The sulphonamides and sulphones were not all given at the same dosage rate, but in amounts calculated on previous experiments in cattle and poultry to give approximately equivalent clinical responses. Table V shows oocyst counts on the first and third/fourth days and the mean (group) weight changes on the sixth day.

It should perhaps be added that the sheep were clinically examined and were grouped as evenly as possible but that the morning after the first dose the higher dosage Sulphamezathine group was considered in the best condition as shown by the initial weights. It is possible, therefore, that less improvement could be expected from this group.

The results set out in Table V show that Mepacrine is more or less without effect in ovine coccidiosis.

TABLE V.

THE TREATMENT OF NATURAL ACUTE COCCIDIOSIS IN SHEEP WITH MEPACRINE AND OTHER TREATMENTS.

Treatment	*Dosage rate*	*No. of Sheep*	*Av. Oocyst Count*		*Mean Initial Weight lbs.*	*Mean Weight Change 6th day*	*Remarks*
			1st day	*3rd/4th day*			
1. Untreated ... (Chlorodyne)	Standard	13	160	190	71·2	+4·6	This group had had 3 days successful treatment before weighing.
2. Mepacrine ...	0·01 gm./kg. b.i.d.	11	40	105	70·9	−1·3	do.
3. Sulphamezathine	0·025 gm./kg.	12	70	7	71·8	+2·5	
4. do.	0·04 gm./kg.	12	27	24	75·6	+5·2	This group was probably the least seriously affected.
5. Sulphadiazine	0·1 gm./kg.	10	300	10	67·4	+7·2	This dosage is 2·5 and 4 times that of sulphamezathine.

NOTE :—The clinical condition of both groups 1 and 2 was in each instance very poor on the 3rd day after treatment began, the Mepacrine treated sheep being by far the worst of all groups—they were no doubt suffering from the effects of the treatment as well as the disease.

DISCUSSION

From the experimental results recorded in this paper it appears that Mepacrine is almost valueless in severe experimental coccidiosis in poultry but that in murine coccidiosis it is rather more active if used at very high dosage rates of 0·15 and 0·25 g. per kg. In severe coccidiosis in sheep even at toxic doses (0·01 g./Kg.) it is inadequate.

This conclusion is supported by the increased oocyst output of Mepacrine-treated sheep, and by the continuation of clinical symptoms including loss of weight as compared with the more substantial improvement in the successfully treated sheep all of which gained in weight.

Most of the evidence recorded in the literature on the value of the drug in cattle depends on the uncontrolled treatment of sporadic cases. As has been mentioned earlier these results have not been spectacular. Most of the cases of which details have been published appear to have been mild, and recovery may have been spontaneous. The evidence of its value in severe bovine coccidiosis is, therefore, unconvincing.

SUMMARY

Mepacrine appeared to be without therapeutic value against severe coccidiosis in birds.

In experimental coccidial infection in mice there was definite evidence of activity at high dosage rates.

In spontaneous acute coccidiosis in sheep even high doses of Mepacrine appeared to be without effect.

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THE NUTRITIVE VALUE OF COLOSTRUM FOR THE CALF

VIII. The Performance of Friesian and Shorthorn Calves Deprived of Colostrum*

By

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INTRODUCTION

Reports published in the past few years suggest that Friesian calves are more easy to rear when deprived of colostrum than those of other breeds. Lundquist and Phillips (1943) in the U.S.A. succeeded in rearing new-born Holstein (Friesian) calves, that had been deprived of colostrum, on a skim milk diet supplemented with vitamins A, C, D and nicotinic acid, whereas similar attempts with Guernsey calves failed. Hansen, Phillips and Rupel (1946), also in the U.S.A. successfully reared Holstein calves, that were known not to have received colostrum, using a skim milk diet supplemented by 25,000 i.u. vitamin A and 250 mg. of ascorbic acid daily. Sulphathiazole, given when diarrhoea occurred, may have influenced their results. Blakemore, Davies, Eylenburg, Moore, Sellers and Worden (1948), while carrying out experiments in this country on the relative importance of antibodies and vitamin A in the prevention of calf scours, found that Ayrshire and Jersey calves were more susceptible to the disease than British Friesian calves.

Most of the calves used in our experiments (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin and Lovell, 1949a; Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin and Lovell, 1949b; Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs and Lovell, 1951) have been of the Shorthorn breed, and the numbers that have survived without colostrum have been small. It was therefore of interest to see whether under similar conditions British Friesian calves would fare any better.

METHODS

Plan of experiment. Twelve bull calves of each of the British Friesian and Shorthorn breeds were used. The twenty-four calves were obtained during the period 12th October 1950 to 9th February 1951, the collection of calves of both breeds being evenly distributed over the period.

*Earlier papers of this series have appeared in Volumes 3 to 5 of the *British J. Nutrition.*

Calves. All the calves were deprived of colostrum and were reared on the synthetic milk (Aschaffenburg *et al.*, 1949a) for 3 weeks. Five farms in the neighbourhood of the National Institute for Research in Dairying supplied the Friesian calves, and the Shorthorn calves were obtained from seven farms. Collection and management of the calves, and the treatment of the scouring calves have been described in earlier experiments (Aschaffenburg *et al.*, 1950).

TABLE I

PERFORMANCE OF BRITISH FRIESIAN AND SHORTHORN CALVES DEPRIVED OF COLOSTRUM

	Friesian	*Shorthorn*
Calves :—		
No. used	12	12
No. died	11	11
Mean birthweight of calves (lb.)*	86·2 ± 3·2	86·7 ± 2·6
Mean age of calves at death (days)*	4 ± 0·7	3 ± 0.4
Mean number of days on which calves scoured before death *	3 ± 0·5	2 ± 0·2
Postmortem findings :—		
Bact. coli Septicaemia	8	10
Bact. coli Fibrinous pleurisy and/or peritonitis with septicaemia	3	1
Bact. coli Non-fatal localised intestinal infection	1	1

* Values with their standard errors.

RESULTS

The results, including the post mortem findings, are given in Table I. Eleven out of twelve calves of each of the two breeds died and there were no statistically significant differences between Friesian and Shorthorn calves in the mean age at death, or the incidence of scouring. Although there was a slight indication that the rectal temperature of Friesian calves was elevated (>102·8° F.) more often than that of Shorthorn calves, this difference was not statistically significant.

DISCUSSION

Under the conditions of our experiment, British Friesian calves do not differ in performance from Shorthorn calves when they have been deprived of colostrum. The divergence of these results from those of others can possibly be explained in part by the conditions under which our experiment was made. Fifty calves had passed through our calf house during the two months before this experiment, and eighty-two were on other experiments while the present one was in progress. It is probable, therefore, that infection in the pens was more intense than in many experiments described by other workers.

SUMMARY

Twelve Shorthorn and twelve British Friesian bull calves were deprived of colostrum and reared for 3 weeks on a standard diet based on dried skim milk.

Eleven calves of each breed died. There were no differences between the two breeds in the mean age of the calves at death or in their performance.

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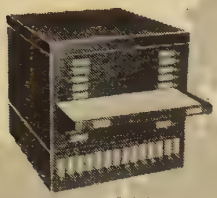

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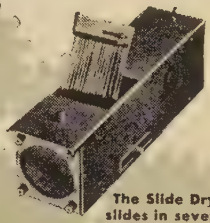

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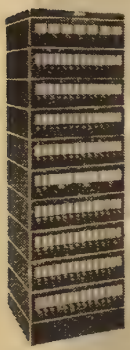

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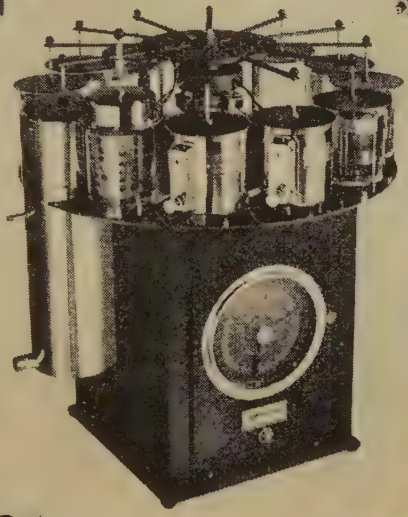

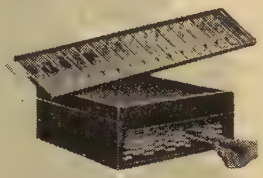

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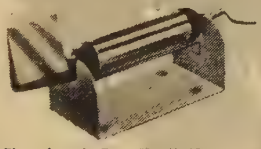

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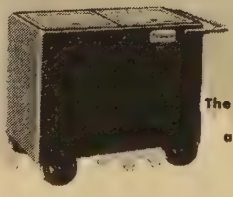

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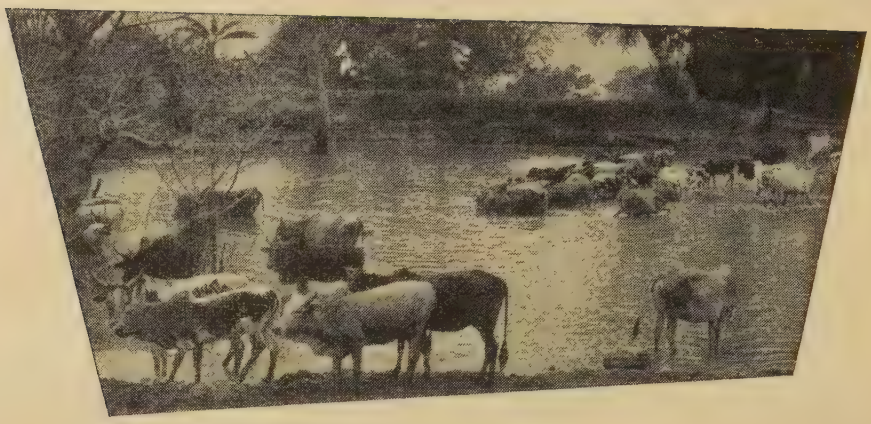

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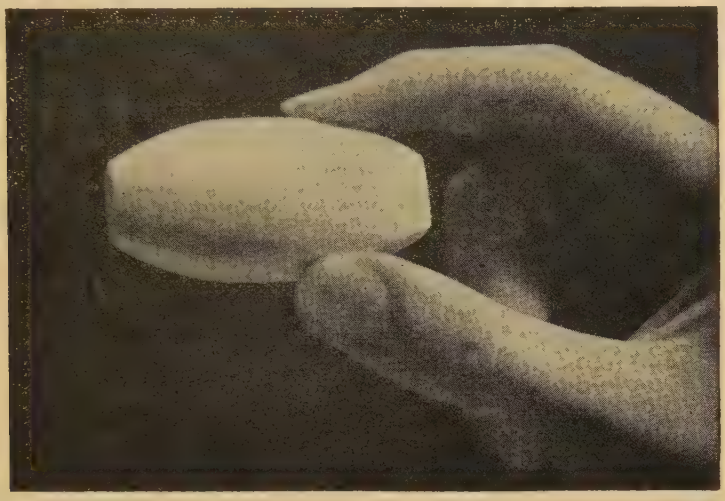

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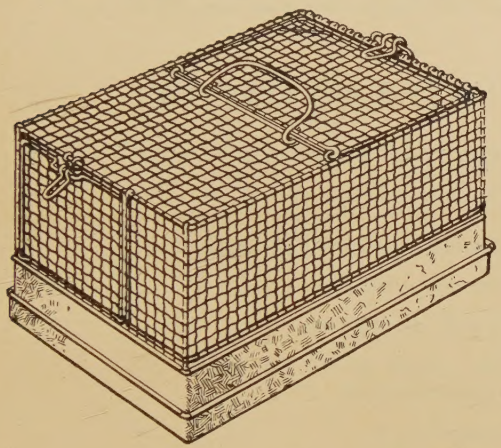

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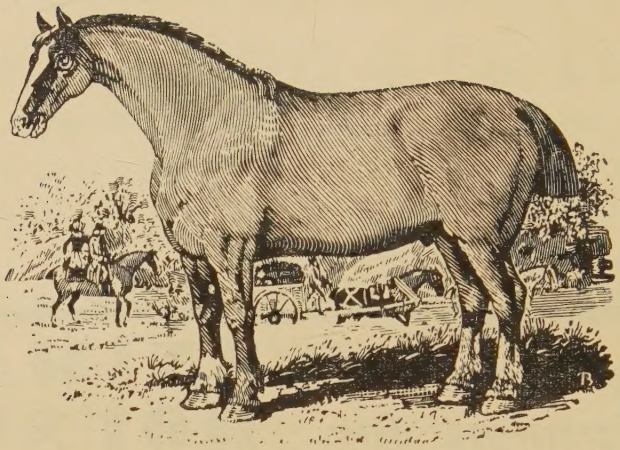

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